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BASAL METABOLIC DATA OF 21 TO 35 YEARS OLD FILIPINO ADULTS

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FOUR TEXT FIGURES

INTRODUCTION

Many excellent reviews on the subject of basal metabolism have been made abroad. Although the first standards for basal metabolism were proposed by Aub and Dubois in 1917,(1) metabolic measurements on infants were reported as early as 1899 in Europe.(2) Comprehensive studies on basal metabolism from infancy up to adulthood have likewise been done in the United States, England and other countries. In the Philippines, until recently, only a few studies have been reported. Fleming(3) in 1923 reported that the average basal metabolic rate of eight male Filipinos was 5.3 per cent less than the Aub and Dubois standards. Sison and Ignacio in 1927(4) reported a study among 76 Filipinos where they found an average basal metabolic rate of 2.8 per cent less than the Aub and Dubois standards. In 1930, Ocampo and co-workers(5) reported their results among 104 normal Filipinos. The 88 males had an average basal metabolic rate of 5.6 per cent less and the 16 females 10.6 per cent less than the Harris Benedict standards.

Knowledge on the energy expenditure of Filipinos is essential for estimating optimal food energy requirements which in turn necessary for appraising the adequacy of available food supplies and estimating food production needs of the country.

For this purpose, a cooperative study was undertaken by three groups: Far Eastern University (FEU), University of the Philippines (UP), and the Food & Nutrition Research Center (FNCR. Guevara *et al*(6) for the FEU group

studied 173 Filipino college students ranging in age from 20 to 33 years; Springer *et al* (7) for the UP studied 200 Filipino subjects ranging in age from 8 to 53 years. In all these studies a uniform method of procedure and reporting of results was agreed upon.

The present study reports on a total of 63 male and 48 female subjects ranging in age from 21 to 35 years taken from the personnel of the National Institute of Science & Technology (NIST) and enlisted men from the Armed Forces of the Philippines (AFP). The Mayo Foundation Standards(8) based on surface area, and the Kleiber's formula(9) on metabolic size, which is calculated as weight in kilograms raised to the three fourths power, were used in the analysis of data.

EXPERIMENTAL PROCEDURE

Subjects were given physical and medical examination to eliminate those suffering from conditions which might alter the basal metabolic rate. Height and weight of the subjects were taken with ordinary light clothing without shoes. The weight was later adjusted to nude weight for the purpose of surface area determination, by subtracting 0.6 kilogram for women and 0.8 kilogram for men which were the figures found in preliminary trials as the average weight of the clothes worn. Our experience shows that relatively large errors in these figures do not significantly affect surface area.

The Benedict-Roth metabolism apparatus which records oxygen consumption was used for our determinations. A preliminary test was made a day or two before the actual test to accustom the subject to the procedure. Subjects who had been adjudged healthy and who were able to adjust to the preliminary trial test were scheduled for the basal metabolism determination. They were instructed to take a light meal the night before the test, to retire early, avoid strenuous physical activity, and omit breakfast till after the test was taken. Subjects were instructed to report directly to our clinic upon rising with a minimum of activity. Basal metabolism determinations were made between 6:30 and 8:00 o'clock in the morning, after subjects had rested in bed and stayed awake for 30 minutes. Two 8-minute determinations were performed on each subject at an interval of about 5 minutes. Tests were made for two consecutive days. The mean of the lower of two determinations on successive days were recorded as the basal metabolic rate value

of the subject.(10) Body temperature and pulse rate were noted prior to the test while the blood pressure was taken after the test.

The basal metabolic rate was expressed as calories per square meter of body surface derived from height and weight using a nomogram,(8) calories per unit of body weight and calories per unit of metabolic size (W (kg.)$^{0.75}$).

RESULTS AND DISCUSSIONS

The results of Guevara *et al*(6) and of Springer *et al*(7) for the same age range; namely, 21 to 35 years are included for a better evaluation of data for this age range. These additional data from 34 males and 22 females from UP and 96 males and 52 females from FEU increased the number of subjects reported here to 193 males and 122 females or a total of 315 subjects.

It will be observed that in both the male and female subjects, the basal metabolic rates per square meter of body surface obtained are generally lower than the Mayo Foundation standards. The differences, however, are all within plus or minus 10 per cent of the standard which is generally considered as the normal range and is in agreement with earlier studies done in the Philippines.(6, 7)

Table 1 also gives the actual oxygen consumption expressed in different units of body size measurements. The mean basal calories per unit of metabolic size obtained in this study was 72.67 ± 6.45 calories for males and 64.72 ± 5.88 calories for females. The standard deviations are higher than the 1.2 calories found by Kleiber.(9) When the basal metabolic rate was expressed per unit of body weight it was found that the standard deviation of the means of both male and female subjects do not exceed the $\pm$ 5 cal. level of deviation.

Further analysis of data presented in Table 1 indicates a close agreement in the results obtained in this study with the two preceding studies. For the male subjects, the mean basal metabolic rates per unit of body surface of the three groups; namely, FNRC, FEU, and UP subjects only differ by about 1 calorie with the FNRC mean of 38.54 cal/m^2/hr. A similar difference of about 1 calorie was also noted when the mean basal metabolic rate is expressed per unit of body weight, while a difference of about 2 calories exists among the mean basal metabolic rates per unit metabolic size of the three groups.

TABLE 1.—*Mean basal metabolic rate data of three groups of subjects.*

	FNRC	FEU	UP	Mean of 3 groups
Males				
Number of subjects	63	96	34	193
Height (cm)	163.90 ±5.78	165.54 ±6.53	162.88 ±5.95	164.54 ±6.18
Weight (kg)	55.74 ±5.95	55.66 ±7.20	53.74 ±6.93	55.39 ±6.78
Basal metabolic rate:				
Deviation from Mayo Stds	±2.26	±1.14	±1.11	±1.50
Calories/sq meter/hour	38.54 ±3.16	38.70 ±3.05	39.96 ±4.06	38.87 ±3.31
Calories/kilogram/day	26.57 ±3.20	27.21 ±2.91	28.33 ±3.62	27.20 ±3.18
Calories/metabolic size/day	72.67 ±6.45	74.19 ±6.67	76.47 ±7.89	74.10 ±6.91
Females				
Number of subjects	48	52	22	122
Height (cm)	154.77 ±6.[illegible]	165.54 ±5.91	152.16 ±5.59	154.62 ±6.19
Weight (kg)	46.38 ±5.00	42.62 ±5.13	46.35 ±5.31	46.06 ±5.08
Basal metabolic rate:				
Deviation from Mayo Stds	±5.96	±5.73	±1.61	±4.14
Calories/sq meter/hour	33.72 ±3.38	36.22 ±3.41	34.81 ±2.54	34.98 ±3.43
Calories/kilogram/day	24.75 ±2.52	26.94 ±3.05	25.40 ±2.42	25.80 ±2.90
Calories/metabolic size/day	64.72 ±5.88	69.75 ±6.22	66.29 ±5.13	67.15 ±6.30

Similar differences of varying significance in the results were noted among the female subjects in the three groups. A difference of 2 to 3 calories was found when the mean basal metabolic rates were expressed as calories per square meter body surface. There was a difference of 1 to 2 calories when basal metabolic rates were expressed as calories per kilogram of body weight, and a difference of 3 to 5 calories when basal metabolic rates were expressed as calories per metabolic size per day.

When basal metabolic rate was expressed per unit of body weight, it was found that the difference between the mean for females and that for the males was not significant at 5-per cent level. However, when surface area or metabolic size was used as the frame of reference, significant differences were obtained between the mean of the female and male subjects.

Table 2 showing the analysis of variances among the data obtained from the three groups, indicate that there is significant difference at 5-per cent level in the mean height of the male subjects. Likewise, the same results can be noted in the mean basal metabolic rate expressed as calories per kilogram body weight and the mean basal metabolic rates expressed as calories per metabolic size. No significant difference is noted in the mean weight and basal metabolic rate expressed as calories per square meter of body surface.

Among the female subjects, the differences are highly significant in the basal metabolic rate expressed as calories per square meter of body surface, calories per kilogram of body

TABLE 2.—*Analysis of variances in the data of FNRC, FEU, and UP subjects.*

	F comp. value	
	Male	Female
Height (cm)	3.0201 **	2.3638 *
Weight (kg)	1.7090 *	0.3215 *
Basal metabolic rate:		
Calories/m²/day	2.3063 *	7.3742 ***
Calories/kg/day	3.5004 **	8.2508 ***
Calories/metabolic size, day	3.4390 **	9.3536 ***
^{f}tab (∞ .05)	(2,190df)2.996	(2,119df)3.077

* No significant difference.

** Significant difference at 5-per cent level.

*** Highly significant difference.

weight and calories per metabolic size. However, no significant differences was noted among the subjects' mean height and weight.

In order to determine where the differences shown in Table 2 lay, tests of significance were done between pairs of study groups (Table 3). Among the male subjects, there is significant difference between the mean height of FEU and UP subjects. Likewise, a significant difference exists between the mean basal metabolic rates expressed as calories per kilogram body weight and calories per metabolic size of UP and FNRC subjects.

TABLE 3.—*Tests for significance between pairs of study groups.*

	^{t} comp value		
	FNRC and FEU	FEU and UP	UP and FNRC
Males—			
Height (cm)	−1.6232 *	2.1218 **	−0.8391 *
Mean basal metabolic rate:			
Calories/kilogram/day	−0.3087 *	1.8093 *	2.4720 **
Calories/metabolic size/day	−1.4238 *	−1.6323 *	2.5564 **
^{t}tab (∞.05)	(157df) 1.960	(128df) 1.960	(95df) 1.983
Females—			
Mean basal metabolic rate:			
Calories/sq.m./day	−3.6804 ***	1.7504 *	1.3389 *
Calories/kilogram/day	−2.8989 ***	2.1150 **	1.0009 *
Calories/metabolic size/day	−4.1555 ***	2.4109 **	1.1453 *
^{t}tab (∞.05)	(98df) 1.987	(72df) 1.996	(68df) 1.997

* No significant difference.

** Significant difference at 5-per cent level.

*** Highly significant difference.

Among the female subjects, there is highly significant difference between the mean basal metabolic rate expressed as calories per square meter body surface, calories per kilogram body weight and calories per metabolic size of the FNRC and FEU subjects. Significant differences also exist between the

mean basal metabolic rate expressed as calories per kilogram body weight and calories per metabolic rate of FEU and UP subjects.

Tables 4a and 4b give the mean basal metabolic rates expressed per unit surface area, weight and metabolic size at age intervals of 21 to 35 years for male and female subjects, respectively. To arrive at average BMR values for subjects 21 to 35 years, data obtained in earlier studies falling within this age group, made by Springer *et al* (7) and Guevara *et al* (6) was included in the study.

TABLE 4a.—*Basal metabolic rate of male subjects values are mean ± S.D.*

Age groups	Number	Mean basal metabolic rate		
		Cal./m^2/hr	Cal./kg/day	Cal./mol. size/day
21	8	39.34 ±3.25	28.77 ±3.25	75.72 ±6.20
22	13	38.64 ±3.92	27.88 ±3.61	74.88 ±8.25
23	18	40.22 ±4.45	28.99 ±4.55	78.32 ±7.64
24	21	38.93 ±2.96	27.69 ±3.29	75.74 ±6.41
25	14	38.36 ±5.47	30.51 ±6.83	74.14 ±4.79
26	24	39.51 ±3.28	27.50 ±3.05	75.54 ±7.26
27	11	38.76 ±3.65	25.92 ±2.21	78.22 ±5.98
28	18	37.94 ±3.37	25.98 ±3.18	71.08 ±7.10
29	6	38.73 ±2.51	24.77 ±5.12	71.68 ±5.08
30	3	37.34 ±5.91	24.42 ±3.57	68.64 ±10.62
31	10	38.21 ±2.68	26.23 ±1.93	72.10 ±4.59
32	11	38.00 ±3.84	26.22 ±3.19	71.30 ±7.78
33	9	33.33 ±2.93	26.52 ±2.47	72.19 ±5.94
34	15	38.99 ±2.82	26.51 ±3.08	73.38 ±6.20
35	12	39.30 ±3.11	23.08 ±4.19	74.52 ±6.98

TABLE 4b.—*Basal metabolic rate of female subjects values are mean ± S. D.*

21	39	34.79 ±5.73	25.88 ±3.11	67.34 ±6.64
22	20	35.58 ±3.16	26.04 ±2.51	67.97 ±5.44
23	16	36.35 ±4.10	27.33 ±3.52	70.38 ±7.61
24	17	34.14 ±3.23	25.08 ±2.10	65.15 ±5.74
25	8	35.48 ±3.85	26.05 ±2.78	67.62 ±7.44
26	8	34.35 ±3.52	24.99 ±2.23	65.25 ±5.82
27	5	34.29 ±1.60	25.82 ±0.60	66.29 ±2.52
28	1	32.70	23.32	61.44
29	2	33.44 ±2.04	22.92 ±2.21	61.56 ±4.93
30	0	±	±	±
31	2	35.98 ±0.71	26.12 ±1.29	68.88 ±2.04
32	0	±	±	±
33	1	34.62	26.88	67.20
34	0	±	±	±
35	3	33.87 ±1.30	23.07 ±2.75	62.83 ±5.13

Figures 1a and 1b show the graphical representation of the data shown in Table 1. Likewise, the graphical representations of data presented in Tables 4a and 4b are shown in Figures 2a, and 2b. No effort was done to smooth out the curves for purposes of constructing standards. It is felt that more data are needed in the same age group as well as in the neighboring age groups before this can be done. There is, however, a noticeable tendency for the curves to go down slightly with increasing age.

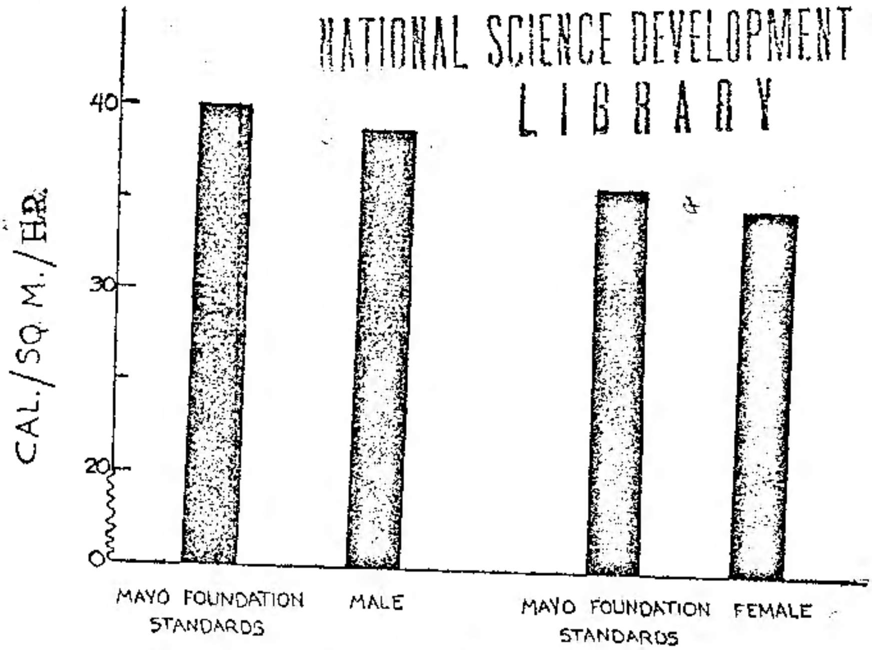

FIG. 1a. Mean basal metabolic (cal./sq. m./hr.) of the subjects as compared to the Mayo Foundation Standards.

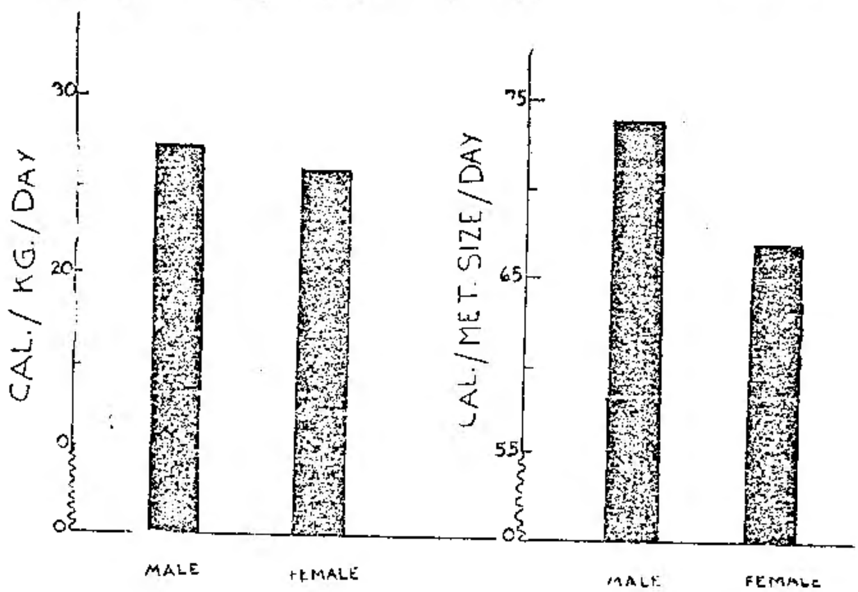

FIG. 1b. Mean basal metabolic rate (cal./kg./day and cal./met. size/day) of male and female subjects.

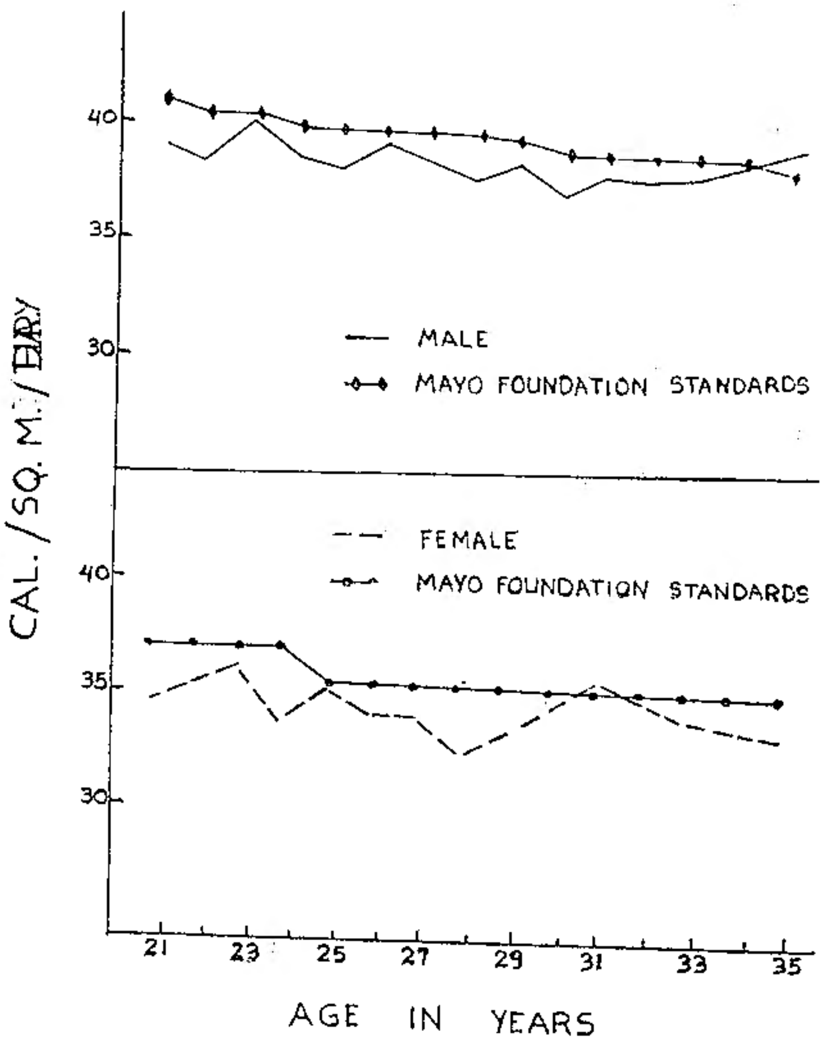

FIG. 2a. Mean basal metabolic rate (cal./sq. m./hr.) of the subjects as compared to the Mayo Foundation Standards.

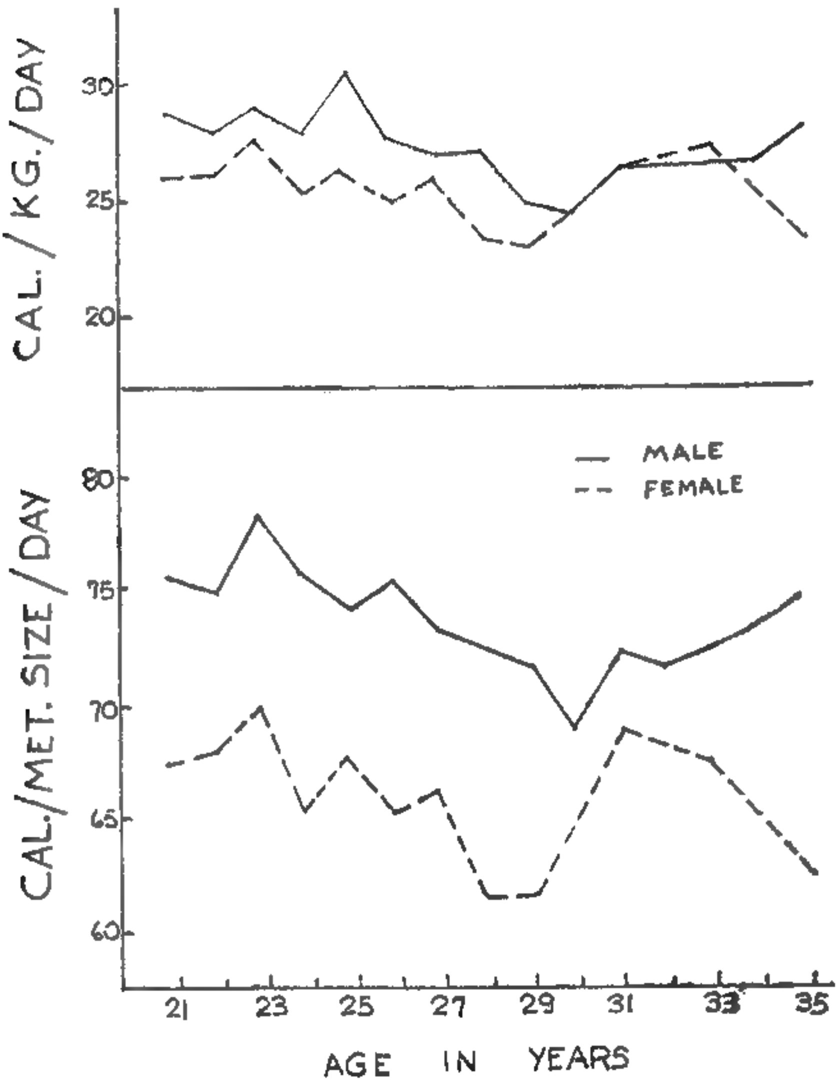

FIG. 2b. Mean basal metabolic rate (cal./kg./day and cal./met. size/day) of male and female subjects.

Figures 1a also shows a graphical comparison between Mayo Foundation Standard and the mean BMR data (cal./sq. m./hr.) from the three local groups. As a whole the Filipino young adult man and woman have a BMR in the order of 3 per cent less than the Mayo Foundation Standard. Difference in the results obtained could be attributed to the difference in the selection of a value for reporting basal metabolic measurements. The BMR values used in the Mayo Foundation Standards were taken from the first BMR test made on the subjects, whereas, in the local studies, the mean of the lower of two duplicate determinations on successive days were taken as the BMR values for the subjects. In a previous study made,(10) we found that the latter method gives slightly lower values than the former. It may be stated therefore, that the basal energy expenditure of Filipino young adults expressed in terms of surface area is more or less the same as that of the Westerners. Similar studies on age groups 7 to 12, 40 to 59, and 60 and above will be made.

SUMMARY AND CONCLUSION

In this study, the basal metabolism data of 193 males and 122 females ranging in age from 21 to 35 years expressed per unit of body surface area, weight and metabolic size are presented and discussed. The data were collected from subjects of this particular study which included personnel from NIST and enlisted men from the Armed Forces of the Philippines. We have likewise integrated in this study, BMR data of subjects within the same age group from studies of Guevara *et al*(6) and Springer *et al*(7) to arrive at average BMR values. The male subjects ranging in age from 21 to 35 years (average 27.46 yrs.) had an average BMR of 38.87 Cal/m^2 hr. or 1.5 per cent less than the Mayo Foundation Standards. The female subjects, ranging in age from 21 to 35 years (average 23.48 yrs.) had an average BMR value of 34.98 Cal/m^2 hr. or 4.14 per cent less than the same standards. As a whole, the Filipino young adult man and woman have a BMR in the order of 3 per cent less than the Mayo Foundation Standards. Considering the differences in the method used in this study and that of Boothby *et al*(8) in selecting BMR values, the basal energy expenditure of Filipino young adults expressed in terms of surface area, is more or less the same as that of the Westerners.

ACKNOWLEDGMENT

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A STUDY OF THE FATTY ACIDS AND LIPIDS OF CLOSTRIDIUM BOTULINUM TYPE B*

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FIVE TEXT FIGURES

Bacterial lipids have a pattern distinct from that of other life forms. They differ in such respects as the absence of sterols, phospholipids low in nitrogen and high in carbohydrate, the lack of the classical lecithins and cephalins, and the presence of a large proportion of free fatty acids some of which are unusual and are absent in other forms of life.

Because of these differences, speculation has been made on the probable relationship of lipids to the enhancement of bacterial virulence,(26) the tolerance to high temperature(7, 16, 42) and to radiation resistance.(27)

Much of the earlier work on bacterial lipids was limited to determinations of "per cent total lipids" for various organisms. An exhaustive review on the subject was given by Kates,(24) O'Leary,(36) and Asselineau and Lederer(5) on the many aspects of the researches conducted. The fatty acid which are the fundamental components of bacterial lipids have been studied mostly to establish the identity and relative abundance in the bacterial cell as a whole. A few microorganisms have been studied for their fatty acid spectra.(4, 6, 17, 18, 19, 21, 23, 25, 27, 33)

This study on the fatty acids and lipids of the vegetative cells of *Clostridium botulinum* 115B was made as an initial step to understand its high resistance to radiation as well as heat and its toxigenicity.

MATERIALS AND METHODS

Reagents.—All chemicals used were of reagent grade. Ether was redistilled and "high purity" hexane was double distilled before use.

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Culture.—*Clostridium botulinum* strain 115B used in this study was obtained from the Quartermaster Food and Development Command for the Armed Forces, Natick, Massachussetts, U.S.A.

The media used as trypticase-peptone glucose (TPG) both containing 50 grams trypticase (BBL); 5 grams peptone (Difco); 5 grams glucose (anhydrous reagent); and 1,000-ml-distilled water. The pH before autoclaving was 6.8. Two- and 5-liter quantities were used to grow the cells, using 10-ml inoculum per liter from a 48-hour TPG growth culture. The inoculum came from a TPG cooked meat broth stock culture which was subcultured twice for 48 hours in TPG to promote vegetative cells and eliminate any lipid or fat contaminant that may be present in the cooked meat medium. Inoculation was done soon after autoclaving or after driving off the excess oxygen to permit anaerobic incubation.

Incubation for 72 hours at 30°C was sufficient for maximum growth of cells without their undergoing sporulation and autolysis. Before harvesting, the culture was checked for purity and sporulation by Gram staining and plating on blood agar and trypticase egg yolk plates and incubating at 30°C aerobically and anaerobically. The cells were centrifuged for 20 minutes and washed free of the medium with distilled water three times in a Sorvall centrifuge. Washed cells were then pooled and lyophilized.

EXTRACTION PROCEDURE

Extraction for lipid class studies.—Lyophilized cells were extracted using the method of Huston and Albro(20) and washed free of nonlipid contaminants by the method of Folch *et al.*(12) The residue was refluxed and extracted for the bound lipids, washed free of nonlipids, and the two extracts were combined and evaporated to dryness under a stream of nitrogen on a 50°C water bath. The residue was weighed, dissolved in chloroform (10 mg/ml) and stored under nitrogen in the refrigerator.

Extraction for fatty acid studies.—Shorter chained fatty acids, because of their volatility were determined as free acids since esters are more volatile and therefore easily lost.(34) The lyophilized cells were ground with chloroform and enough 0.2 N sulfuric acid to keep the mixture acidic. Then it was extracted twice with the acidified chloroform. The extracts

were combined and evaporated to dryness under nitrogen at room temperature. The residue was then taken up in hexane for introduction into the gas liquid chromatograph.

For the longer chained fatty acids, extraction was carried out with lyophilized cells using Huston and Albro's method.(20)

Extraction for the free lipids.—Lyophilized cells weighing from 1 to 2 grams were shaken for 1 hour in 100 ml of acetone. It was then filtered through Whatman No. 1 filter paper and the residue was shaken for 2 hours in 100 ml of chloroform-methanol (2:1 v/v). It was again filtered and shaken with the same mixture for another 2 hours. This was filtered and the residue was shaken for 1 hour in 100 ml of chloroform-methanol (1:1 v/v). All extractions were carried out at room temperature to prevent alterations of lipid structures.(15, 28) After filtration the residue was set aside for bound lipid extraction while the extracts were pooled and evaporated to dryness under nitrogen. The residue which consisted of free lipids was dissolved in ether.

Extraction for bound lipids.—After the last solvent extraction the residue was refluxed for 2 hours in a nitrogen atmosphere with a minimum amount of 2 N–KOH using glass beads. The resulting solution was acidified with 6 N–HCL and extracted four times with 200 ml of chloroform. The extracts were pooled and evaporated on a water bath at 50°C under a stream of nitrogen. The residue was dissolved in 9 ml of chloroform-methanol (2:1 v/v), washed free of nonlipid contaminants and taken up in ether.

Separation of the free fatty acids.—Column chromatography as described by McCarthy and Duthie(32) was employed. Their method was modified(27) to recover the phospholipids, by evaporation to dryness of the methanol fraction, dissolving the residue in chloroform and adding dilute hydrochloric acid to dissolve the potassium formate. The chloroform phase was separated and the residue containing the phospholipid was taken up in ether. The ether fractions containing the glycerides and the free fatty acids were also evaporated separately on a 50°C water bath and the residues were taken up in ether.

Preparation of methyl ester derivatives.—A "total" lipid fraction was formed by combining the glyceride, phospholipid and bound lipid fractions. This was refluxed for 1 hour in a nitrogen atmosphere with 100 ml of methanol-benzene-con-

centrated sulfuric acid (17:2:1). After stopping the reaction with water it was made alkaline with aqueous NaOH and extracted for the methyl esters with 200 ml of double distilled hexane. The extract was evaporated to dryness under nitrogen and taken up in hexane (10 mg/ml).

The methyl esters of the free fatty acids were also prepared using the same procedure.

Purification of methyl esters.—Thin layer chromatography was used to purify the methyl esters of both "total" acid and free fatty acid. The spotted samples and reference standard were run by ascending technique in hexane-ether-acetic acid (90:10:1) for 25 minutes at room temperature. Vacuum aspiration was used to remove the purified methyl esters which was eluted with chloroform, evaporated to dryness on a 50°C water bath and taken up in hexane (10 mg/ml).

Hydrogenation of methyl esters.—To detect which component of the methyl esters were unsaturated, the microhydrogenation procedure of Farquhar *et al*(11) was modified by the use of hexane to prevent transesterification.(39)

Gas liquid chromatography of fatty acids.—Chromatographic instrument used was F & M high efficiency chromatograph. The operating conditions were set as follows:

Column: For higher fatty acids
- 6′ × ⅛ O.D. 15 per cent EGS on chromosorb P 60–80 mesh
- Column temperature: 190°C
- Carrier gas: helium   Flow rate: 40 ml/min.
- Injector temperature: 240°C
- Detector temperature: 215°C
- Sample size: 3 ul.

Column: For lower fatty acids
- 6′ × ⅛ O.D. 20 per cent FFA phase on Chromosorb P 60–80 mesh
- Column temperature: 175°C
- Carrier gas: helium   Flow rate: 40 ml/min.
- Injector temperature: 225°C
- Detector temperature: 200°C
- Sample size 3 ul.

The EGS column had a theoretical plate number of 1.135 measured from the methyl stearate curb and an HETP of 0.16. The efficiency of separation of 18 and 18.1 was used as the index to measure the efficiency of the EGS column which was equivalent to 0.82.

Identification and quantitation.—Three ul of the samples were chromatographed in triplicate. Standard mixtures were chromatogrammed in between sample runs for determining accurate relative retention times and carbon number since the retention times changed as the column was used longer. The following standard volatile acids in 3-ul quantities were used 1:0, 2:0, 3:0, 4:0, and 6:0. Standard methyl esters were 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 21:0, 22:0. Identification of the component peaks was based on relative retention times of known esters or by the carbon number method of Woodford and Van Gent.(44)

Thin layer chromatography.—To characterize the different lipid classes the thin layer chromatography technique as described by Mangold(30) was used. Standards used were caphalin, a mixture of mono-, di-, and tripalmitin, oleic acid and a standard mixture of methyl esters of fatty acids.

The plates were prepared using a Desaga-Brinkman applicator, air-dried and activated by heating in an oven 110° to 120°C for 2 hours. Lipids were characterized by developing in hexane-ethyl ether-acetic acid (90:10:1 v/v/v).(30) After vacuum aspiration and elution, phospholipids and glycolipids were developed with chloroform; methanol; water (70:22:3 v/v/v),(30) and the glycerides with skellysolve B; ethyl ether (70:30 v/v).(38) The indicators used were iodine vapors for detection of all unsaturated lipids and some saturated nitrogenous lipids,(30, 31) 0.2-per cent ninhydrin in butanol for aminophosphatides,(8) 0.2 per cent 2′ 7′ dichlorofluorescein in 96-per cent ethanol for the saturated and unsaturated nonpolar lipids,(30) fuchsin-sulfuric acid for plasmalogens, iodine-benzidine and 50-per cent sulfuric acid for glycerides,(8) Bial's reagent and diphenylamine for glycolipids,(41, 43) and chromic-sulfuric acid solution followed by charing for the detection of all organic matter.(30, 35)

RESULTS AND DISCUSSION

Fatty acids.—Using gas liquid chromatography (GLC) tentative identification was made on 28 out of 37 fatty acids found in the free fatty acid fraction (Figure 1, Table 1) and on 20 out of 26 fatty acids detected in the total lipid fraction (Figure 2, Table 2). Identification was based on comparison of relative retention times of the unknown peaks with those of

TABLE 1.—*Free fatty acid composition of Clostridium botulinum 115B grown at 30°C in TPG medium.*

Peak number	Rel. ret. time Tr./Tr	Compound [1]	Carbon number	Per cent composition
1	0.019	8:1	8.4	0.01
2	0.022	8:2	8.5	tr [4]
3	0.032	un [2]	8.8	0.01
4	0.03[illegible]	9:0	9.0	0.02
5	0.047	9:1	9.3	0.01
6	0.053	9:2	9.5	0.01
7	0.073	10:0	10.0	0.08
8	0.0[illegible]7	un	10.9	0.07
9	0.112	11:1	11.3	tr [4]
10	0.137	12:0	12.0	0.69
11	0.164	12:2	12.5	0.06
12	0.189	13:0	13.0	0.07
13	0.213	13:0 cy [3]	13.3	0.13
14	0.228	un [2]	13.6	0.15
15	0.26[illegible]	14:0	14.0	15.14
16	0.308	14:1	14.3	1.60
17	0.342	un [2]	14.8	0.89
18	0.364	15:0	15.0	0.41
19	0.426	15:1	15.4	0.17
20	0.449	un [2]	15.7	0.09
21	0.524	16:0	16.0	44.96
22	0.586	16:1	16.5	6.23
23	0.6[illegible]4	un [2]	16.7	4.03
24	0.713	17:0	17.0	0.80
25	0.781	17:0 cy [3]	17.3	4.90
26	1.000	18:0	18.0	4.57
27	1.120	18:1	18.5	6.11
28	1.26[illegible]	18:2	18.7	1.09
29	1.3[illegible]0	un [2]	19.1	0.72
30	1.[illegible]08	19:1	19.[illegible]	1.09
31	[illegible]	un [2]	19.8	0.47
32	2.1[illegible]0	20:0	20.0	0.20
33	2.[illegible]1	20:2	20.8	2.15
34	3.104	21:0	21.0	2.17
35	3.3[illegible]	21:1	21.[illegible]	0.78
36	3.813	21:2	21.8	0.09
37	4.511	un [2]	22.[illegible]	0.53

[1] Number of carbon atoms in acid, number of double bonds

[2] un=unidentified

[3] cy=cyclopropane

[4] tr=trace—less than .01

standards, rechromatography, the reaction of the unknown to hydrogenation and their "carbon number" from plots of equivalent chain lengths *vs.* retention times of normal saturated methyl ester standards. The retention times of normal saturated fatty acid methyl esters from C_8 to C_{22} and unsaturated fatty acids C 18:1, C 18:2, and C 18:3 which were used as standards are given in Table 3. Tentative identification is given in "carbon numbers" with retention times relative to caproic acid.

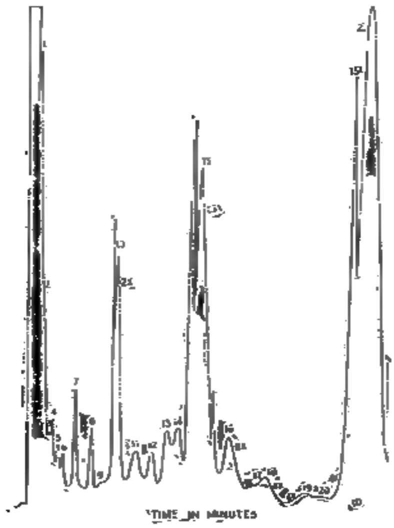

FIG. 1. Gas chromatogram of methyl esters free fatty acids of *C. botulinum.*

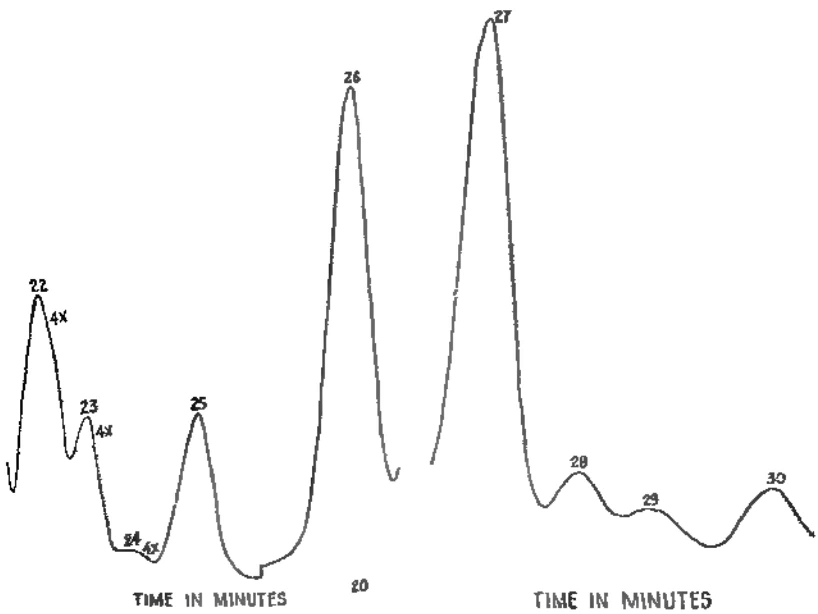

FIG. 1. Continued

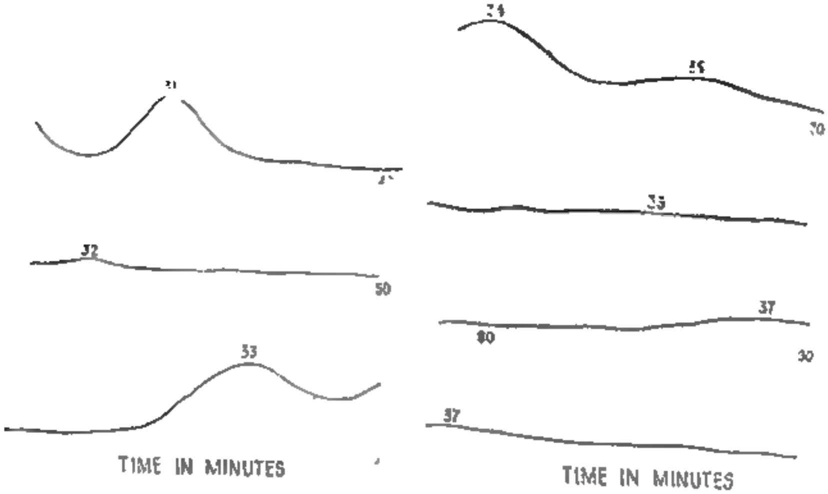

Fig. 1. Continued

Table 2.—*Total fatty acid composition of Clostridium botulinum 115B grown at 30°C in TPG medium.*

Peak number	Rel. ret. time Tr./Tr.	Compound [1]	Carbon number	Per cent composition
1	0.016	8:0	8.0	0.32
2	0.031	8:1	8.5	1.00
3	0.043	un [2]	8.9	0.38
4	0.049	9:1	9.2	0.17
5	0.058	un [2]	9.6	3.07
6	0.066	10:0	10.0	0.49
7	0.077	10:1	10.5	1.14
8	0.103	11:0 cy [3]	11.3	0.55
9	0.114	11:1	11.6	0.61
10	0.131	12:0	12.0	9.05
11	0.160	12:3	12.9	0.57
12	0.181	13:0	13.0	1.97
13	0.223	un [2]	13.6	0.52
14	0.254	14:0	14.0	19.94
15	0.313	un [2]	14.8	5.55
16	0.356	15:0	15.0	2.21
17	0.386	15:0 cy [3]	15.3	0.40
18	0.347	un [2]	15.6	0.26
19	0.501	16:0	16 [illegible]	32.88
20	0.575	16:1	16.4	0.51
21	0.710	17:0	17.0	0.42
22	0.780	17:1	17.2	3.69
23	0.914	un [2]	17.8	4.05
24	1.000	18:0	18.0	2.65
25	1.103	18:1	18.7	7.79
26	1.474	18:3	19.6	0.87

[1] Number of carbon atoms in acid; number of double bonds
[2] un=unidentified
[3] cy cyclopropane

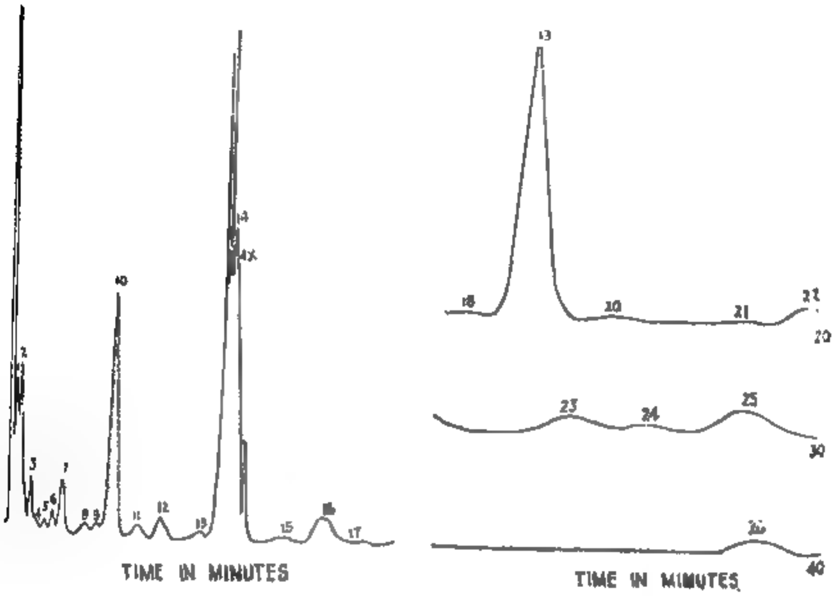

FIG. 2. Gas chromatogram of methyl esters of total fatty acids of *C. botulinum* 115 B.

GLC demonstrated the presence of fatty acids with short chain carbon atoms from C_1 to C_8 (Figure 3, Table 3), the most predominant of which was caproic acid. These compounds have long been known to occur in bacteria and have been detected in almost all species studied.(24)

The free fatty acid fraction of bacteria have been reported to range from 2.1 to 44 per cent (2, 3, 4, 24) which is large compared to cells of other forms. Some unusually high percentages reported have been attributed to lipolysis during extraction.(15, 28)

The overall pattern for this organism included straight chain saturated, unsaturated and cyclopropane acids with a complete fatty acid spectra from C_1 to C_{21} (Tables 1, 2, and 3).

The normal saturates made up 69 per cent of the free fatty acid fraction and 68 per cent of the total lipid fraction while the normal unsaturates constituted 19 and 15 per cent of both fractions respectively. Palmitic acid was predominant in the straight chain saturated fraction with 32.88 per cent in the total lipid (Table 2, Figure 2) and 44.96 per cent in the free fatty acid fraction, (Table 1, Figure 1). Literature reports this

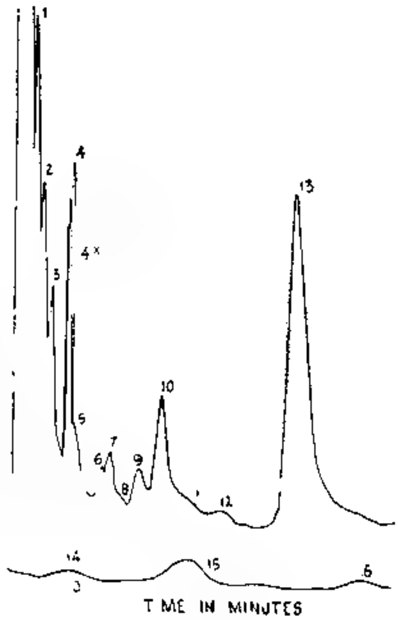

FIG. 3. Gas chromatogram of volatile fatty acids of *C. botulinum* 115B.

TABLE 3.—*Relative retention times of standard fatty acid methyl esters.*

Compound [1]	Rel. ret time Tr. Tr.	Carbon number
8 0	0 016	8 0
9:0	0 042	9 0
10:0	0 067	10 0
11·0	0 099	11 0
12:0	0 129	12 0
13 0	0 202	13 0
14:0	0 280	14 0
15·0	0 3[illegible]2	15 0
16:0	0 530	16 0
17 0	0 708	17 0
18·0	1 000	18 0
18:1	1 080	18 3
18 2	1 301	18 6
18 3	1 682	19 5
19 0	1 810	19.0
20:0	2 591	20 0
21:0	3 700	21.0
22 0	5 290	22 0

[1] Number of carbon atoms in acid: number of double bonds

acid to be the most characteristic constituent of fatty acids occurring more frequently and usually in larger amounts than any other saturated acid found in bacteria.(10, 14, 23) Law(25) found in his studies that in 14 out of 16-gram-negative bacteria, C_{16} saturated straight chain fatty acid accounted for 35.8 to 54.8 per cent of the fatty acids. In this study, the second predominant acid was myristic acid which formed 19.98 per cent of the total lipid (Table 2, Figure 2) and 15.14 per cent of the free fatty acid fraction (Table 1, Figure 1). Myristic acid represents 24 per cent of the fatty acid of *Clostridium perfringens* (14) and is the major saturated acid found in *Clostridium botulinum* 33A.(33) It has also been reported in other bacterial species.(9, 20, 40) Also present were normal saturates with an odd number of carbon atoms from C_9 to C_{21} except C_{19}. These have been found in *Clostridium butyricum*,(24) *Clostridium botulinum* 33A(33) and other bacterial species.

Monoenoic acids made up the bulk of the normal unsaturated acids in this organism with C_{18}; 1 being the major one followed by C_{16}:1. Both were reported in *Clostridium butyricum*(24) and *Clostridium botulinum* 33A.(33) Palmitoleic and octadecenoic acids are considered predominant in microorganisms.(9, 17, 22) Analysis also revealed the presence of unsaturated higher chain fatty acids such as 17:1, 18:2, 19:1, 20:2, 21:1, and 21:2.

Cyclopropane acids are known to occur in both gram positive and gram negative species. *Clostridium botulinum* 33A contained four tentatively identified cyclopropane acids, C_{11}, C_{13}, C_{15}, and C_{17},(33) while *Clostridium butyricum* had C_{13}, C_{15}, C_{17} and C_{19}.(24) Cyclopropanes have also been detected in *Escherichia coli*, *Lactobacillus* sp., *Agrobacterium tumefaciens*, *Bacillus subtilis*, and *Streptococcus* sp.(37) Four cyclopropane acids were tentatively identified with the 115B strain, i.e. C_{11}, C_{13}, C_{15}, and C_{17} which follows closely strain 33A.

The lipids of *Clostridium botulinum* 115B contained essentially the same fatty acids as those of *Clostridium botulinum* 33A except for the absence of the fatty acid tentatively identified as an 11 carbon numbered compound which was present in trace quantity and the fatty acid tentatively identified as containing 21 carbon atoms. The quantitative distribution of the various fatty acids differed somewhat.

Lipids.—The free lipid and bound lipid (total lipid fraction) of *Clostridium botulinum* 115B was resolved into its component lipid classes on thin layer plates. Resolution (Figure 4)

FIG. 4. Thin layer chromatogram of lipid classes on silica gel. Solvent hexane-ethyl ether-acetic acid, 90:10:1 v/v/v. Development time, 25 min. (*1*) oleic acid, (*2*) methyl esters, (*3*) phospholipids, (*4*) mono-, di-, and tri-palmitin, (*5*) lipid sample of *Clostridium botulinum* 115B. (left to right.)

indicated that it contained hydrocarbons which moved along with the solvent front, free fatty acids, di- and triglycerides (Figure 5) and phospholipids. After elution and further resolution, the phospholipid fraction indicated the presence of plasmalogens and aminophosphatides. Plasmalogens have been found so far only in anaerobic bacteria such as *Clostridium butyricum,*(6, 13) *Clostridium botulinum* 33A,(33) and some species of the rumen bacteria.(1) The appearance of light brown spots after spraying with chromic-sulfuric acid and before charring the chromatograms indicated that unsaturated fatty acids were present in the free fatty acid fraction and the phospholipid fraction.

The presence of glycolipid was also detected using diphenylamine and Bial's reagent.(41) About 70 per cent of the extractable lipids of *Clostridium perfringens* was found to

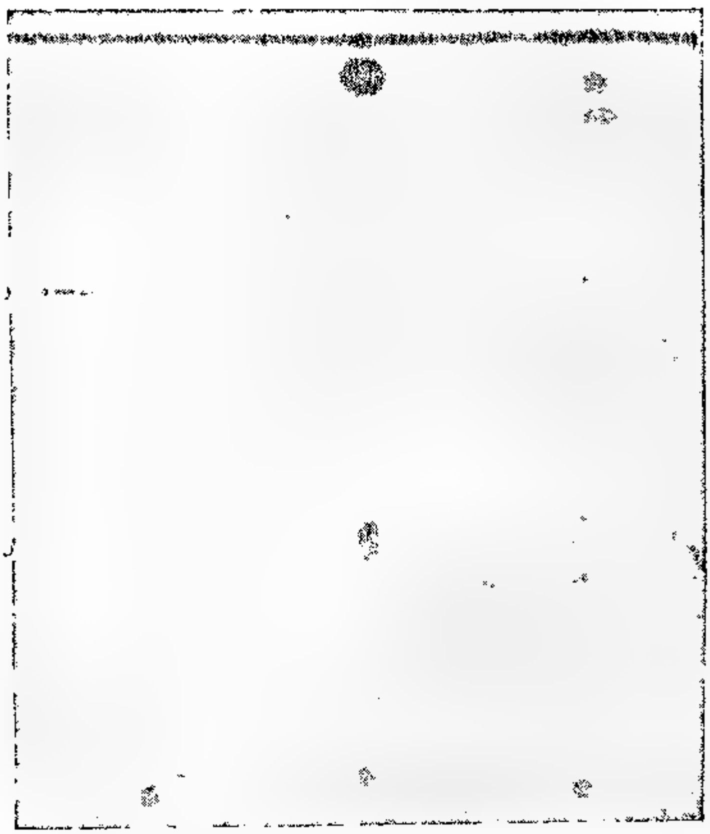

FIG. 5. Thin layer chromatogram of phosphoglyceride fraction on silica gel. Solvent Skellysolve B: ethyl ether, 70:30 v/v. Development time: 20 min. (*1*) phospholipid, (*2*) mono-, di-, and tri-palmitin, (*3*) phosphoglyceride fraction of *Clostridium botulinum* 115B.

consist of phosphatides and glycolipids,(24, 29) glycolipids have also been characterized in *Eubacteria*.(24)

SUMMARY

The lipid composition and fatty acids of the vegetative cells of *Clostridium botulinum* 115B grown in TPG medium at 30°C were studied. The volatile fatty acids were determined as free acids, the higher fatty acids were converted into methyl esters. Both were characterized using gas liquid chromatography.

Hydrocarbons, di- and triglycerides, free fatty acids, phospholipids and glycolipids are the component lipids of this organism. The overall fatty acid pattern included straight chain saturated, unsaturated and cyclopropane acids with a complete spectra from C_1 to C_{21}.

The most abundant volatile fatty acid was caproic acid. The major portion of the higher fatty acids was made up of normal saturates with palmitic acid predominating followed by myristic acid. Monoenoic acids made up a large group of the normal unsaturated acids with C_{18}: 1 being found in the greatest proportion.

The lipids of *Clostridium botulinum* 115B contained essentially the same fatty acids as those of *Clostridium botulinum* 33A; however, the quantitative distribution of the various fatty acids differed somewhat.

ACKNOWLEDGMENT

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STUDIES ON PHILIPPINE MEDICINAL PLANTS, IV[1]

ALKALOIDS OF ALSTONIA MACROPHYLLA WALL (LEAVES AND FRUITS)[2,3]

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The continued interest on the alkaloids of *Alstonia macrophylla* Wall has been sustained by reports on the structure of two more alkaloids from the stem bark of this plant. Macrosalhin (4) $C_{21}H_{27}O_2N_2^+$ as a quaternary base, represents a new skeletal type in the alkaloid field. Macralstonidine, $C_{41}H_{48}O_3N_4$, ((11) on hydrolysis gives a macroline derivative and N(.)-methyl-sarpagine and formaldehyde.

The alkaloids present in the roots (6, 7) are: macralstonine, macralstonidine, demethoxy-alstophylline, alstophylline, pleiocarpamine, villalstonine and quarternary bases. Those obtained from the stem bark are: villalstonine, macralstonine, alstophylline, pleiocarpamine, N(.)-methyl-2,16-dihydro-akuammicine, macrosalhin and macralstonidine.(4, 5, 11) The structures of these alkaloids have been confirmed.

In the previous paper (6) the alkaloids from the leaves were obtained by an extraction process that was followed for the stem bark. (5) In this present report, the alkaloids from the leaves and the fruits were recovered by a modification of the process of "sequential extraction" of Hultin. (3)

The color reactions of the alkaloids from the roots of this plant have been studied. (7) Solutions of the alkaloids were treated separately with solutions of ceric ammonium sulfate in phosphoric acid, ferric ammonium sulfate in phosphoric

[1] For paper III, see Manalo, G. D., Nat. Appl. Sci. Bull. 20 (1967) 225–235.

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[3] Read at the First Post War Philippine Science Convention held at Manila, July 7-20, 1968.

[4] Professor of Industrial Pharmacy, College of Pharmacy, retired.

acid and ferric ammonium sulfate in sulfuric acid. The colors change rapidly and the final color reaction is often missed. The impurities in the alkaloid mixtures which give different colors interfere in the reaction of the main alkaloid.

Fansworth *et al*(1) were able to identify a majority of the 63 alkaloids from the *Catharanthus* species under study. The alkaloid samples were chromatographed on thin-layer plates of silica gel G and developed with solvent systems: ethyl acetate-absolute alcohol (3:1); n-butanol-glacial acetic acid-water (4:1:1) and methanol. The chromatoplates were sprayed with a solution of ceric ammonium sulfate in phosphoric acid.

The alkaloids were classified into eight chromogenic types of response differing in the color changes observed. The chromogenic comparison of the alkaloids is summarized in a table. The R_f values of the alkaloids in the three solvent systems used are also tabulated.

Mattocks, A. R.(8) has described a spectrophotometric method of determination for pyrrolizidine alkaloids. The samples, after oxidation, were treated with modified Ehrlich reagent. An improved oxidizing reagent(9) has been tried on retrorsine, senecionine, monocrotaline, laisocarpine, heliotrine and rosmarinine. Absorbance was measured at 563 $m\mu$ for these alkaloids except rosmarinine (562 $m\mu$).

The chromatographic mobilities of 23 synthetic steroid hormones on silica gel and alumina plates have been examined.(2) Three solvent systems used were: I, benzene-acetone (4:1); II, benzene-methanol (9:1); III, Bush LB21/A85, Light petroleum (b.p. 100–120°C)-benzene-glacial acetic acid-water (67:33:85:15). The chromatograms were visualized with three spray reagents which gave different colors. The colored spots were also observed under ultra-violet light. The ΔR_m values for several functional groups of steroids indicated a correlation between the structure of the steroids and their chromatographic behavior.

The chromogenic response of the alkaloids from the roots, stem, bark, leaves and fruits to ceric ammonium sulfate spray reagent is presented in this paper. The chromatograms were developed on thin-layer plates of silica gel G with acetone-benzene-ammonia (32:32:1). The colors observed were comparable to those obtained with the alkaloids from the *Catharanthus* species.

MATERIALS AND METHODS

The different parts (roots, stem bark, leaves, and fruits) of *Alstonia macrophylla* Wall were obtained from trees growing on Mt. Makiling, Los Baños, Laguna. The powdered air-dried materials (roots, stem, bark, and leaves) were percolated with alcohol and finally exhausted with alcohol containing 1-per cent hydrochloric acid. The combined percolates were concentrated separately under reduced pressure.

The seeds which are small and very flat with deep brown hairs were removed from the double follicle, long slender fruit. This remaining follicle is henceforth designated as "pods." This term is used throughout the paper. From a sample of the fruit weighing 333 gm, the seeds recovered were 18 gm. This whole weight of the seeds was exhausted with petroleum ether by repeated maceration process. The resinous pods were cut into small pieces and also exhausted with petroleum ether in the same manner. The residues from this extraction were macerated separately with acidified (1-per cent hydrochloric acid) alcohol to extract the alkaloids. The solvent was distilled off under reduced pressure.

The concentrated alkaloidal extract from the seeds was macerated in water and the resinous precipitate which separated out was filtered off. The filtrate was basified with ammonia (pH 10) and shaken with several portions of ether until all the alkaloids have been extracted. The ether extracts were washed with distilled water and then dried with anhydrous sodium sulfate. The ether was distilled off and hexane was added to precipitate the alkaloids. Separate samples of the precipitate and the hexane solution were tested on chromatoplates of silica gel G.

An acidic aqueous solution of the alkaloids from the pods was prepared in a similar manner. This aqueous solution was shaken first with benzene until exhaustion and followed with chloroform. The residual solution was then basified with ammonia (pH 10) and exhausted first with ether and then shaken with chloroform. The solvents were separately recovered from the extracts. The benzene concentrate was chromatographed through basic alumina (Fisher). The chloroform concentrate from the acidic aqueous solution was passed through a column of basic alumina (Fisher) while that from the basic solution, through neutral alumina Woelm

(Activity II). Benzene and chloroform (in succession) were the eluents in each of the columns. The alkaloids from the ether concentrate were precipitated with hexane.

The alkaloids from the leaves were obtained in a different manner. The concentrated extract of the leaves was triturated with Hyflo Supercel (1:1) and macerated with water. The combined aqueous extracts were then shaken with portions of benzene and after exhaustion, with portions of chloroform. The residual aqueous solution was then basified with ammonia (pH 10) and shaken with ether. The benzene and the chloroform extracts were concentrated before passing through columns of basic alumina (Fisher). The ether extracts, after recovery of the solvents, were evaporated to dryness and dissolved in the minimum amount of chloroform. For the recovery of the alkaloids, benzene and chloroform were used as eluents.

A Desaga spreading apparatus was used for preparing the thin-layer plates. A slurry of 15-gm silica gel G (E. Merck Darmstadt) in 30-ml water was spread on five (20 × 20 cm) glass plates. With ceric ammonium sulfate spray reagent, however, a slurry of 30 gm of silica gel G in 30-ml water was used for the five plates. Acetone-benzene-ammonia (32:32:1) mixture was the developing solvent.

Paper chromatography was performed in the same manner as in the previous reports.(6, 7) The chromatogram was developed with methyl ethyl ketone saturated with water and detected with Dragendorf's alkaloidal reagent. The spots were also examined under ultraviolet light using a long wave Black lamp (UVL–21).

The same procedure for the alkaloids of the *Catharanthus* species(1) was followed in the preparation of the plates and the spraying with ceric ammonium sulfate reagent. Dragendorf's alkaloidal reagent was used to confirm the position of the alkaloidal spots on the chromatoplates.

The mass spectra of the samples from the leaves, seeds and pods were measured and interpreted through the kindness of Dr. M. Hesse[5] of the University of Zurich, Switzerland.

[5] The generous assistance of Dr. M. Hesse is gratefully appreciated. Sincere thanks are also extended for the samples of pleiocarpamine and villalstonine.

RESULTS AND DISCUSSION

The residue from the petroleum ether extract of the seeds is 3.18 per cent and that from the pods, 2.52 per cent. The fatty material from the seeds is yellow which deposited some crystal upon standing at room temperature, while that from the pods is greenish yellow. The crude alkaloid (precipitated with hexane) from the seeds is pale yellow and amorphous which melted at 170° to 175° C (decomp., uncorrected). The yield from the seeds is 0.04 per cent and that from the pods is 0.23 per cent.

The chromatographic behavior of the alkaloid samples from the different parts of the plant is summarized in Table 1. The relative proportion of the alkaloids present in the corresponding extracts is indicated by the relative intensity of the color of the spots produced when the plates were sprayed with Dragendorf's alkaloidal reagent. The results showed that the main constituent of the seed is the alkaloid with R_f 0.60. The other alkaloid, R_f 0.40, is present in trace amounts.

TABLE 1. —*Chromatographic behavior of the alkaloid samples.*

Samples	Thin-layer plates (silica gel G)	Paper chromatograms
	Rf	*Rf* (fluorescence)*
Seeds	0.40	
	0.60	0.60 (yellow)
Pods		
ether extract	0.20	0.02 (orange with blue rim)
	0.37	0.36 (blue-violet)
	0.57	0.47 (green)
	0.69	0.70 (red)
		0.84 (red)
Leaves		
benzene extract	0.30	0.[illegible]8 (green)
	0.47	0.70 (red)
	0.57	0.90 (blue)
	0.87	
chloroform extract	0.11	0.31 (orange with green rim)
	0.30	0.70 (red)
	0.17	
ether extract	0.47	0.08 (orange)
	0.57	0.11 (blue)

*The fluorescence was observed with a long wave Black Lamp (UVL-21)

The yellow crude alkaloid residue from the pods contains compound, R_f 0.57 in the largest proportion. A white amorphous precipitate separated from the benzene eluate of the chloroform extracts of the acidic solution of the total alkaloids. This sample decomposed without melting at 205° C and gave a spot on the chromatoplate, R_f 0.20.

A large proportion of the alkaloids of the leaves dissolved in water. This same behavior is exhibited by the alkaloids of the roots. Acidulated water was used to exhaust the alkaloids from the extract of the leaves. Hyflo Supercel removes efficiently the plant pigments from the extract of the leaves.

The chromatograms of the different organic solvent extracts from the solution of the total alkaloids of the leaves indicated the presence of various alkaloids in different proportions. The chromatoplates showed that alkaloids with R_f 0.47 and 0.57 are present in the largest proportions. From the aqueous solution of the total alkaloids, benzene extracted in the same proportion, compounds with R_f 0.30, 0.47, and 0.57. The presence of trace amounts of alkaloids with R_f 0.70 and 0.87 was also indicated. Chloroform extracted mostly alkaloids with R_f 0.30 and 0.47 and traces of the compound with R_f 0.11.

The paper chromatograms showed that benzene extracted from the aqueous solution of the total alkaloids appreciable amounts of the compound with R_f 0.48 and 0.70 and traces of the compound with R_f 0.90. The main constituent in the chloroform extract of the aqueous solution of the alkaloids was the compound with R_f 0.70.

The color reagent, ceric ammonium sulfate in phosphoric acid, produced beautiful colors rapidly changing as it was sprayed on the chromatograms for the *Alstonia* alkaloids. The color reactions are comparable to those exhibited by the *Catharanthus* alkaloids. (1) There are alkaloids which gave colors, especially after 24 hours, different from the changes that were observed in a particular type of chromogenic response for the *Catharanthus* alkaloids. The occurrence of any given alkaloid in the different parts (roots, stem bark, leaves, seeds, and pods) is indicated by the color changes of the spots produced by the extracts from the corresponding part of the plant. To illustrate: an alkaloid giving a crimson red color is present in the pods, leaves and roots; in negligible amounts in the seeds and undetected in the stem bark. The extracts from the pods, roots, and stem bark produced a lavender spot. An alkaloid producing a blue spot was obtained from extracts of the pods, leaves, roots and stem bark. The relative amounts of these alkaloids (as verified by Dragendorf's alkaloidal reagent) vary in these different parts of the plant.

The color reactions of the alkaloid samples together with their corresponding R_f values on thin-layer plates are shown

in Table 2. An alkaloid (Sample 4 from the leaves), although present only in small amounts, could be easily recovered. The identity of this compound as demethoxy-alstophylline could not be ascertained. This alkaloid has been isolated from the roots. Sample A from the pods behaved in the same manner as Sample 1 from the leaves and Sample B may possibly be the same as the alkaloid from the seed.

TABLE 2.—*Chromogenic response of the alkaloid samples to ceric ammonium sulfate spray reagent.*

Samples	Thin-layer plates (silica gel G)	Color of the spots
	R_f	
Alstophylline	0.60	blue rapidly fading then brown
Macralstonidine	0.57	lavender
Macralstonine	0.65	blue rapidly fading then brown
Pleiocarpamine	0.35	pink to pale orange then yellow
Villalstonine	0.53	grayish green then brown
Leaves		
Sample 1	0.20	crimson red developing rapidly an irregular orange yellow center then gray.
Sample 2	0.30	deep orange developing rapidly a red rim to greenish yellow then deep purple
Sample 3	0.34	orange to pink orange then orange red.
Sample 4	0.70	orange red with irregular yellow center then orange
Seeds	0.53	grayish yellow to greenish yellow then yellow.
Pods		
Sample A	0.20	same color reaction as Sample 1 from the leaves.
Sample B	0.54	irregular yellow with green rim to greenish yellow then yellow brown.

The mass spectra (10) of the sample from the seeds indicated the presence of small amounts of pleiocarpamine. The main component has the molecular weight, 354. Intensive fragmentation peaks were not observed. "Peaks at m/e 225 and between 150 and 200 are in favor of ajmalicine or sarpagine type structure."

The sample (white amorphous solid decomposed without melting at 205° C) from the pods gave a crimson red color with ceric ammonium sulfate spray reagent. This was found to be a mixture of four main alkaloids with molecular weights, 322, 338, 354, and 368. "One of the compounds could be pleiocarpamine (main peaks at m/e 322, 263, and 180). The other three are very difficult to analyze. Macroline (M^+ 338) could not be under the mixture because of the lack of its typical fragmentation peaks."

Pleiocarpamine (in very small amounts) was also indicated by the mass spectra of Sample 2 from the leaves. This sample has a melting point of 215 to 218° C (decomp., uncorrected) and gave a deep orange finally deep purple color with ceric ammonium sulfate spray reagent. "The most predominant compound has the molecular weight of 338 and is not identical with macroline because of lack of the typical fragment ion peaks. The compound contains a—$COOCH_3$ group and an unsubstituted indole dihydroindole chromophore."

The above results indicate that these alkaloids from the seeds, pods and leaves are new compounds not yet isolated from the roots and the stem bark. Although they are of the 'monomer' indole type, not one of them is alstophylline which was first isolated from the stem bark nor demethoxy-alstophylline which was first isolated from the roots. Pleiocarpamine is present in the seeds, pods and leaves and in the stem bark and roots as previously reported.(4, 6)

Further studies on these new compounds would require more samples from these different parts of this plant. Additional data and chemical degradation are necessary for further elucidation of the structure of these new alkaloids. Pleiocarpamine may be recovered from alkaloid (C) of the leaves. The major alkaloid (R_f, 0.57) from the pods can be purified and its mass spectra determined. Pharmacologic screening of these compounds is also suggested.

The most recent communications(10) concerning the experience (at the University of Zurich, Switzerland) with the alkaloids of the stem bark of *Alstonia macrophylla* Wall from two different drugs both collected from the Philippines revealed the following information:

> In one we found 1. villalstonine, 2. macralstonine, 3. macralstonidine and in the other, 1. villalstonine, 2. pleiocarpamine, 3. macralstonine and no macralstonidine. But the second isolation gave us a lot of other unknown alkaloids which had not been isolated till now. Maybe the alkaloid content changes during the seasons and/or depends on the age of the trees, we do not know.

The above findings on the alkaloids in the stem bark have a very important bearing on the occurrence of alkaloids in the roots, leaves, and fruits of this plant. A careful analysis of the results from various experiments including a possible comparison of the pharmacological action of the alkaloids in pure or mixture forms would be necessary for a careful evaluation of the economic possibilities of *Alstonia macrophylla*

Wall. It is also believed that the results of these studies on the different parts of this plant would be useful in the consideration of this drug in a future pharmacopoeia and/or formulary of the Republic of the Philippines.

ACKNOWLEDGMENT

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The author wishes to extend sincere gratitude and appreciation for the continued interest and assistance of Dr. Manfred Hesse of the University of Zurich, Switzerland. Sincere thanks are also extended to Miss Helen Africa for her technical assistance and to friends at the College of Pharmacy, and the National Research Council of the Philippines who have in one way or another helped in the pursuance of this research.

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A DITERPENE FROM TINOSPORA RUMPHII BOERL *

By RAMON A. ACEVEDO, ALFREDO C. SANTOS, AND PRIMITIVA PARATAO

FIVE TEXT FIGURES

A confidential documented data on cases of cancer said to have completely regressed or cured by treatment with an ointment prepared from a crude coconut oil extract of *Tinospora rumphii* Boerl (Tag. Makabuhay) stems and *Bryophyllum pinnatum* (Lam.) Kurz. (Tag. Katakataka) leaves turned over by the heirs of the late Dr. Sofronio Tagle, a physician-botanist, to the Araneta University for verification, led the present authors to undertake chemical studies on the two plants.

In a survey of the literature Bacon(2) in 1907, reported that the bitter aqueous extract of the stem of a supposed *Tinospora crispa* Miers does not contain an alkaloid, and the bitter principle was glycosidal in nature, Stangl(10) in 1914 reported the presence of two alkaloids, tinosporin and tinosporidin in a supposed *Tinospora crispa* Miers. However, according to Merrill(7) *Tinospora crispa* Miers is not found in the Philippines and their material was wrongly named by Fernandez-Villar [Novis appl. (1880) 8]. Feliciano(5) in 1921, stated that the bitter principle of *Tinospora reticulata* Miers is glucosidal in nature. In 1939, R. Paris(8) reported that the bitter principle of *Tinospora crispa* Miers was picroretin, a white nonhygroscopic powder. Beauquesne(3) in 1941 reported the presence of waxes, a small quantity of hydrocarbon and an impure substance considered to be phytosterol. Chatterjee and Ghosh(4) in 1960, isolated from all parts of *Tinospora cordifolia* Miers, a bitter principle, tinosporin, m.p. 184°, $C_{20}H_{22}O_6$ or $C_{21}H_{24}O_7$, which showed in the UV spectrum a maximum near about 210 mμ (ϵ 6700) an absorption attributed to a mono-β-substituted furan, and confirmed by the NMR (peaks at —113 and —62 cps.) and the IR peaks at 1506 and 875 cm^{-1}. The carbonyl region of the IR spectrum showed two bands at 1715 and 1745 cm^{-1} attributed to two

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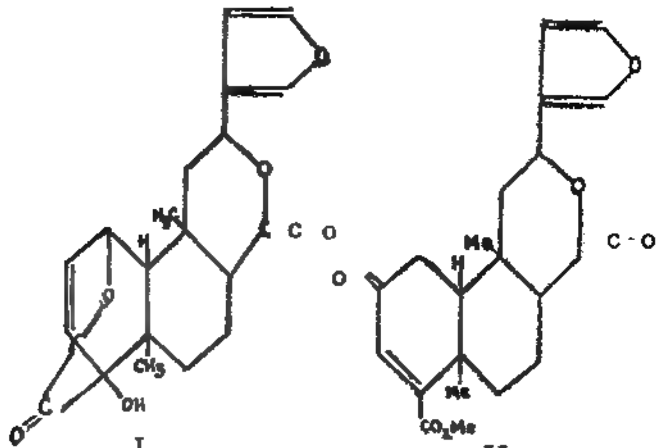
δ-lactones as in columbin (I). Two hydroxyls were reported present. Other compounds not fully characterized was isolated by Palnikar and Paranjpe and by Siddique *et al* were reported in the survey of literature by Chatterjee and Ghosh in



I
COLUMBIN.

II
TINOPHYLLONE

1960. The isolation(6) and the chemical structure (1) of tinophyllon (II), another diterpene from a Philippine Menispermaceæ has also been reported.

As far as *Tinospora rumphii* Boerl itself is concerned, we have not come across any chemical work dealing with the isolation of its constituents.

Preliminary tests—using the common method of separation for the alkaloids and other groups of chemical plant constituents on the different parts of the two plants (roots, stems, leaves, and flowers), failed to give any definite chemical compound in pure form which might be tried for anticancer activity. However, from the stems, hanging roots, ground roots and tubers of *Tinospora rumphii* Boerl, we are able to isolate a pure crystalline compound for which we propose the name *tinosporan.* From the elementary analysis, a molecular formula of $C_{20}H_{22}O_6$ has been derived and which is supported by the mass spectrum (M^+358). The Liebermann-Burchard test indicate a diterpene (II).

Tinosporan is chromatographically uniform. It gives only one spot on Silica gel G in the following solvent systems: ether (Rf 0.31), ethyl acetate (Rf 0.575), benzol + ethyl

acetate + acetone (15:35:50) (Rf 0.58), cyclohexane + ethyl acetate + acetone (15:35:50) (Rf 0.525) petroleum ether + ethyl acetate + acetone (10:4:50) (Rf 0.60).

The UV spectrum (Fig. 1) of tinosporan gave a maximum of 210 mμ indicating a furan ring which is supported by the

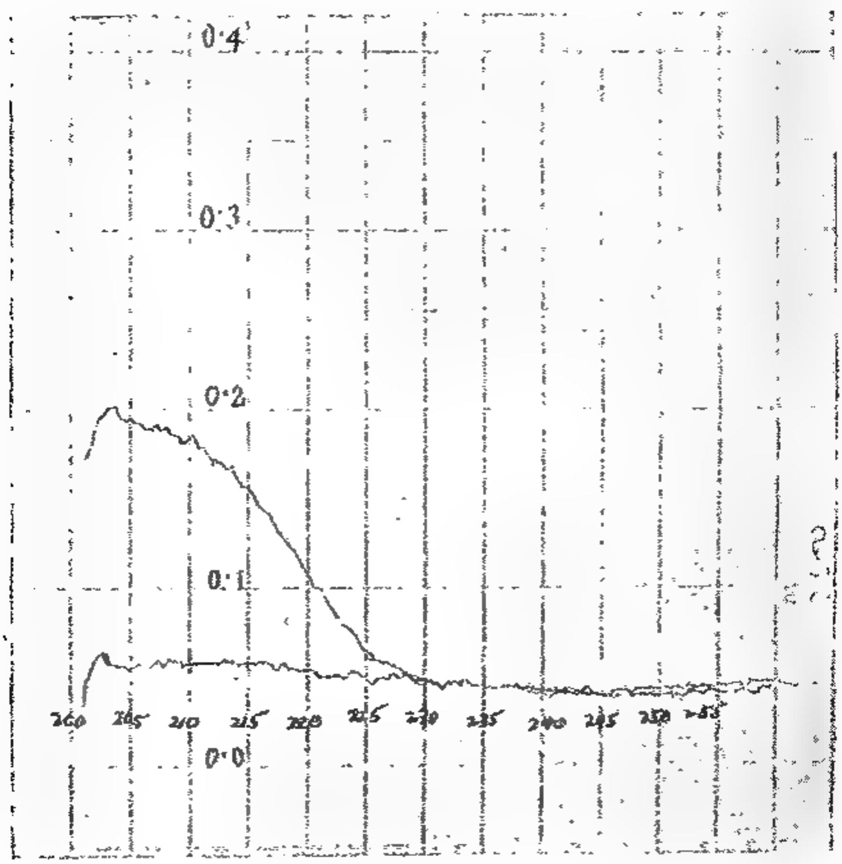


FIG. 1. UV spectrum of tinosporan.

IR (Fig. 2A, 2B) peaks at 1503 and 879 cm^{-1}. The IR shows also absorption at 3500 cm^{-1} indicating a hydroxyl and at 1750 cm^{-1} corresponding to a lactone. The NMR (Fig. 3) showed two methyl groups at τ8.9 and τ8.72, a hydroxyl at τ6.47, a multiplet attributed to a cyclic, nonconjugated —C = C— at τ4.4 — 4.9, two α — H of a β — substituted furan at τ2.48 and one β — H of the furan ring at τ3.53. The mass spectrum of tinosporan gave peaks at m/e 358 [M +], 340 (M–H_2O), 314 (M–CO_2), 296, 246, and 231.

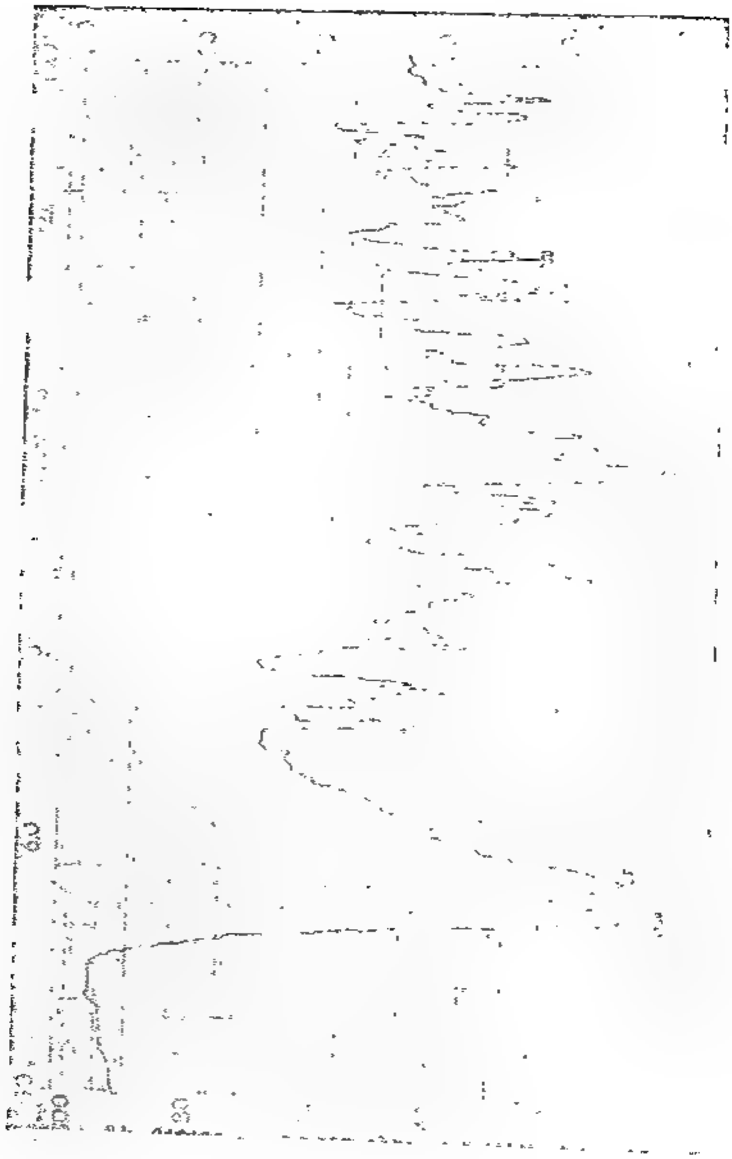


FIG. 2a. IR spectrum (KBr) of tinosporan.

From the foregoing consideration it appears that the diterpene tinosporan must be very closely related to columbin.

Isolation of tinosporan. One hundred eighty grams of air-dried and powdered stems, roots and tubers of *Tinospora rumphii* Boerl, were extracted in a Soxhlet apparatus with hexane to

FIG. 2b. IR spectrum (KBr) of tinosporan.

EXPERIMENTAL

remove fats and waxy substances. The defatted material was dried in a vacuum oven and extracted continuously again this time with ether, until there was no more separation of crystals in the flask. The crude greenish-yellow crystals were purified by washing with warm (70°–80°) distilled water, dried, and washed with petroleum ether until the washings are colorless.

The now still yellowish crystals were washed with small amount of ether until the washings are colorless. The crystals were dried in the vacuum oven and finally recrystallized from ethanol several times until 0.18 g. colorless, glistening needles are obtained.

Yield = 0.1 per cent.

Properties of tinosporan. The compound crystallized from methanol in the form of long, slender and colorless rods with an average dimension of 1 micron in diameter and about 4 to 5 micra in length. Melting point (Kofler block) 180–181° $[\alpha]_D = +38°$ (C – 1, acetone). Tinosporan is soluble in ethanol, methanol, acetone, chloroform, ethyl acetate, slightly soluble in ether and vegetable oils, almost insoluble in water, and most hydrocarbon solvents. *Salkowski reaction:* The chloroform layer was colorless, and the acid layer was red. Liebermann-Burchard test: pink, red, brown.

An elementary analysis[3] of tinosporan: Found 66.90 per cent C, 6.4 per cent H, 26.88 per cent O. Calculated for $C_{20}H_{22}O_6$ 67.04 per cent C, 6.14 per cent H, 26.8 per cent O.

SUMMARY

A diterpene, tinosporan, m.p. 180–181°. $C_{20}H_{22}O_6$, has been isolated from the stems, roots and tubers of *Tinospora rumphii* Boerl.

Considerations of the spectral data—UV, IR, NMR, and MS show that tinosporan must be very closely related to columbin.

ACKNOWLEDGMENT

We are indebted to Drs. Gertrudes Aguilar-Santos and R. T. Aplin for their valuable assistance, and for the measurements of UV, IR, NMR, MS at Oxford, England.

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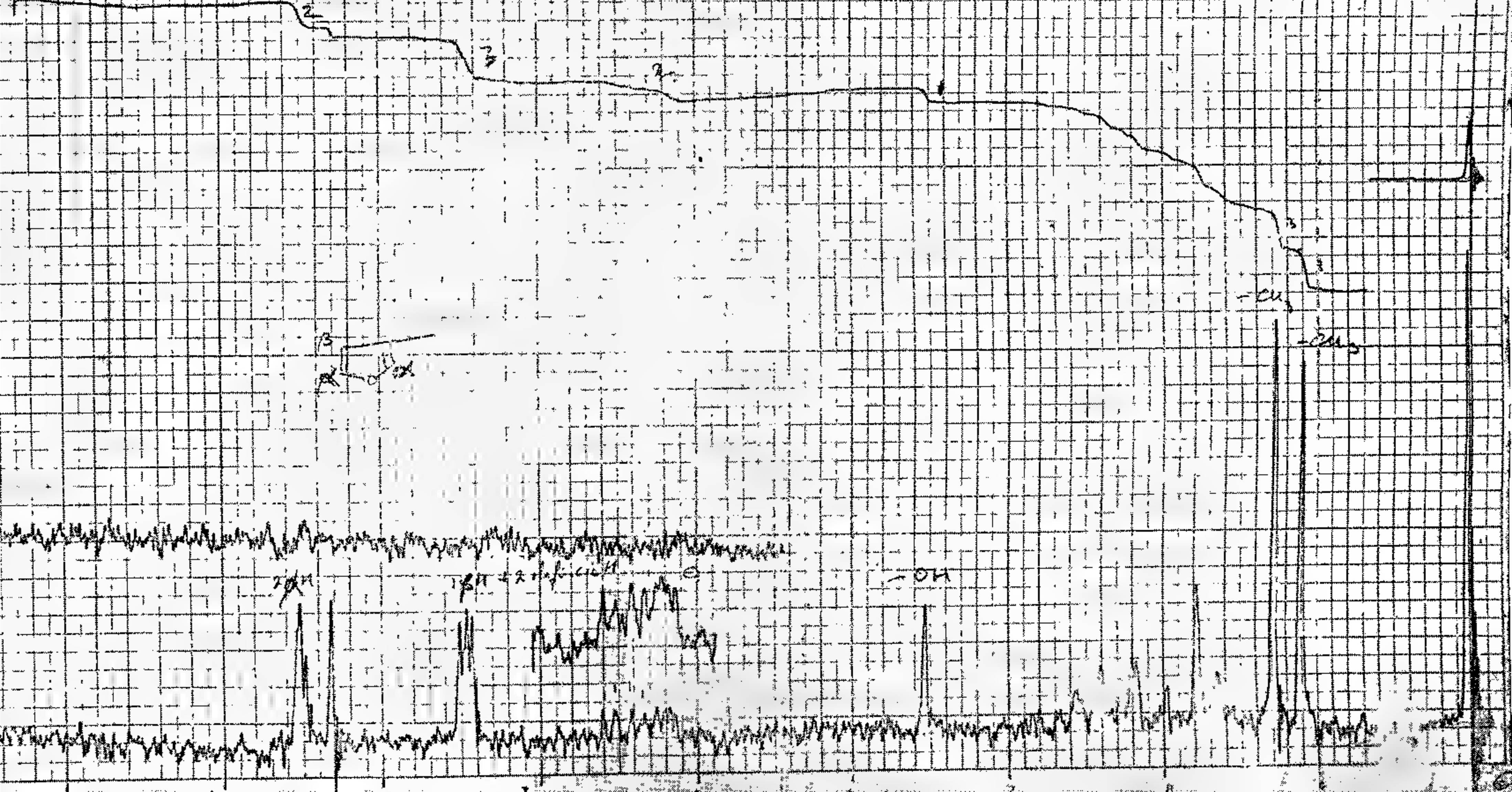
-OH

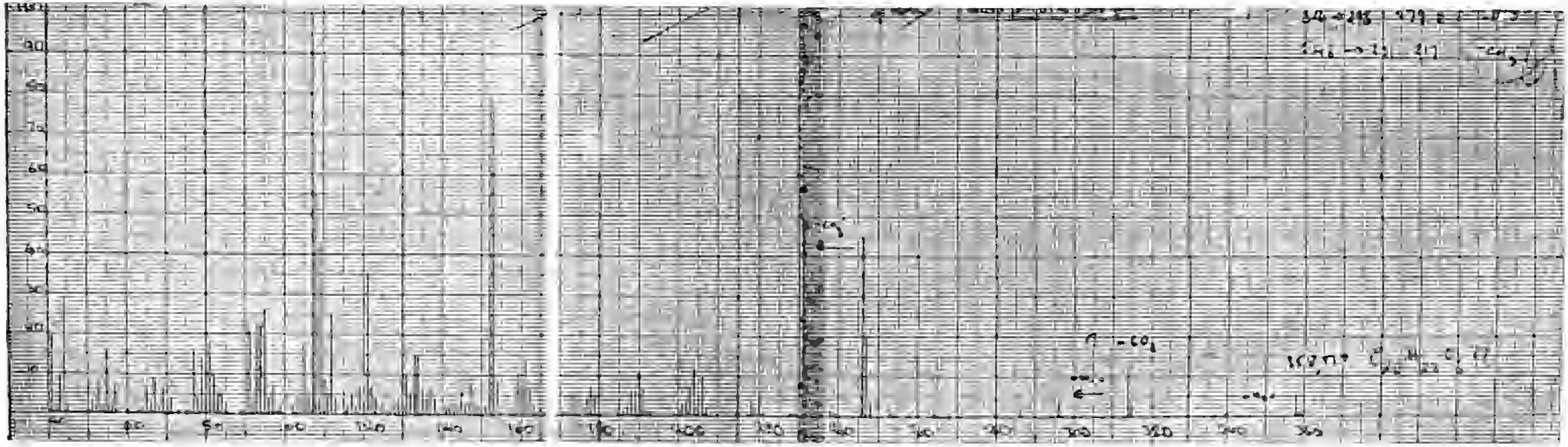

FIG. 4. MS of tinosporan.

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PHYSIOLOGICAL AND CHEMICAL STUDIES FOR THE IDENTIFICATION OF PHILIPPINE MINT

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TWO PLATES AND THREE TEXT FIGURES

Although two species of *Mentha* were reported in the Philippines by Merrill,(7) only *M. arvensis* L. is included in the lists of Philippine flowering plants by Quisumbing(8) and Steiner.(15) The plant is cultivated for medicinal and ornamental purposes and for use as a flavoring. Present-day taxonomists at the Philippine National Herbarium are of the opinion that there is only one species growing throughout the country and that the plant does not flower under Philippine climatic conditions. This is somewhat supported by the fact that only vegetative specimens of Philippine mint or yerba buena, labelled *M. arvensis* L. are found in the Philippine National Herbarium, the U. S. National Herbarium, and the Gray Herbarium. The correct name of Philippine mint or yerba buena prior to this study was therefore open to doubt. This study was undertaken to find out its correct identification.

MATERIALS AND METHODS

Cuttings from one plant growing in the botany garden of the University of the Philippines at Quezon City were grown under three photoperiodic treatments: 9-hour days, 18-hour days, and 9-hour days supplemented with 2 hours of light in the middle of the dark period.

Herbarium specimens of the flowering plants were prepared and sent to the U. S. National Herbarium, the Gray Herbarium, and the A. M. Todd Company at Kalamazoo, Michigan.

Plants were grown in the field under natural daylengths and under interrupted nights. The oil from vegetative and flowering tops was extracted with steam as described in the NF XII (1) and its physical constants were determined. Samples of the oil were sent for analysis to the Takasago Perfumery Company in Tokyo and to the A. M. Todd Company.

Stolons were sent to the A. M. Todd Company for growing under a natural temperate climate.

RESULTS AND DISCUSSION

The plant flowers under long-day conditions (Plate 1, fig. 1), bearing small, white flowers in terminal slender spikes (Plate 1, fig. 2). It is reported by Merrill(6, 7) and Quisumbing(8) that the plant is a native of Europe and was introduced by the Spaniards in the Philippines. It is therefore to be expected that yerba buena is a long-day plant.

The species of *Mentha* are very variable, forming hybrids very readily and they have been crossed and recrossed until hundreds of the vegetatively propagating progeny have been described and very unsatisfactorily defined.(2, 3, 4) According to Clapham *et al*(3) several escape from cultivation and several others are introduced to other continents so that the hybrids are often found away from their parents and the parentage of many of them is in doubt.

M. arvensis and closely related strains bear flowers in axillary whorls,(3, 4) not in terminal spikes as produced by the Philippine mint plants that were induced to flower. Using available systematic keys to the genus *Mentha*(3, 4) was not entirely satisfactory but it could be seen that Philippine mint is related to *M. spicata* L. as pointed out by the associate curator of the U. S. National Herbarium.

M. spicata and its hybrids exhibit a wide variability in chromosome number(3) and in the chemical constituents of their volatile oil.(5, 12, 13) The physical constants of the oil of yerba buena resemble those of the oil from *M. rotundifolia* (L.) Huds. as reported by Shimizu(10) and those of the oil of *M. spicata* L. ($2n = 24$) as reported by Shimizu and Ikeda.(12) The oils are all dextrorotatory and show a maximum UV absorption at 260 mμ indicating that the principal component is an α, β–unsaturated ketone (Table 1). Analyses of the oil from vegetative plants by preparative gas chromatography, NMR, and IR and UV absorption confirmed piperitenone oxide as the major constituent (Figs. 1, 2, 3). Piperitenone oxide or rotundifolone, $C_{10}H_{14}O_2$, is the principal component of *M. rotundifolia* and has been shown by Reitsema(9) and Shimizu (10, 11) to be 1, 2–epoxypulegone or 1-methyl–4–isopropylidene–1, 2–epoxy-cyclohexanone–3 (I).

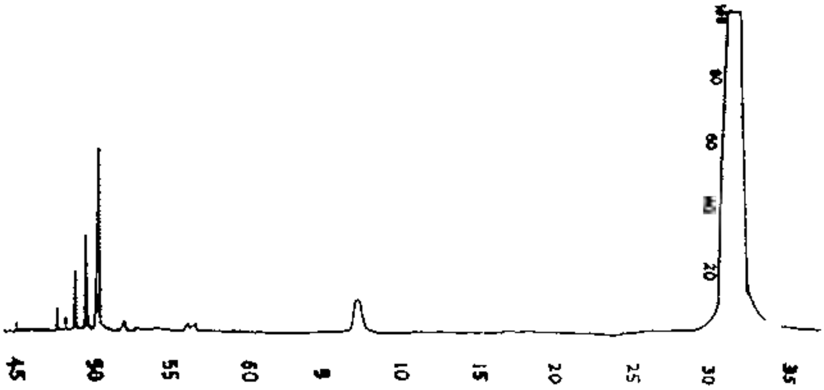


FIG. 1. Preparative gas chromatogram of the oil of Philippine mint.

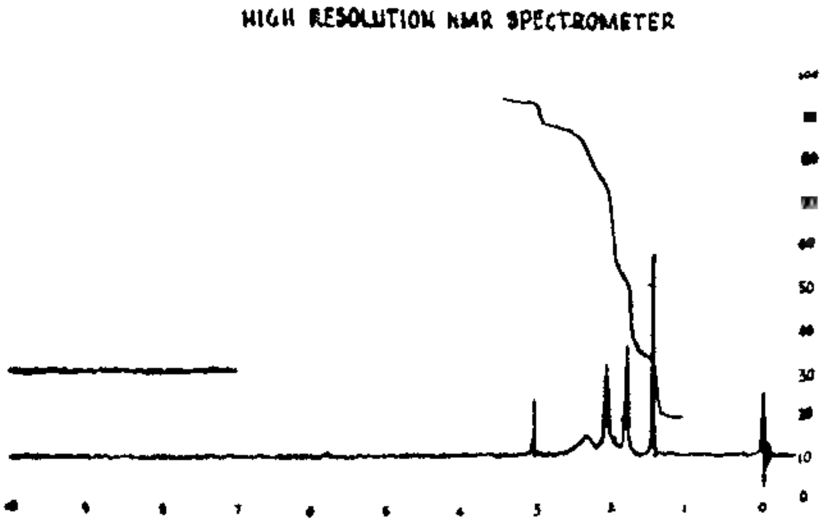


FIG. 2. NMR spectrum of piperitenone oxide from the oil of Philippine mint.

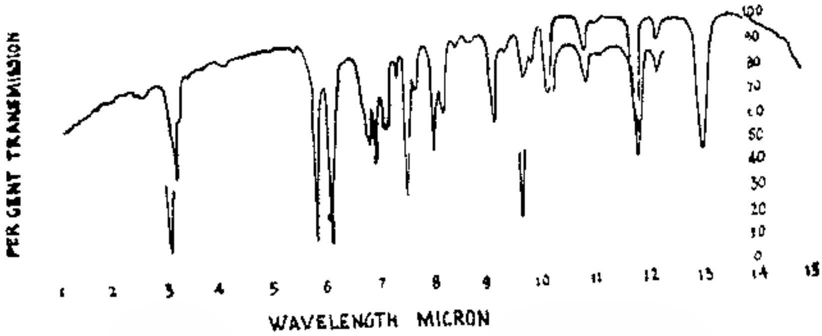
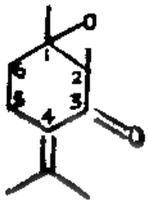


FIG 3. IR absorption spectrum of piperitenone oxide from the oil of Philippine mint. (UV absorption: λ_{max}) 255 mμ ϵ = 7,890 in cyclohexane.)



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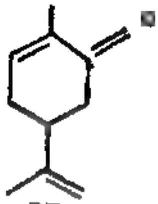
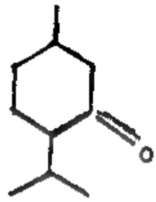


II



III

Since it has oxygen atoms attached to the carbon atoms at positions 2 and 3 of the *p*-menthane ring, it may be regarded as one of the intermediates between the carvone (II) (2-oxygenated-*p*-menthanes) and the menthone (III) (3-oxygenated-*p*-menthanes) series. Piperitenone oxide has also been isolated by Shimizu and Ikeda (12, 13) from strains of *M. spicata* L. (2n = 24) cultivated in Japan but introduced from Europe. They got F_1 clone of *spicata* (2n = 36) type having (+)–piperitenone oxide as a principal component by crossing *M. rotundifolia* [2n = 24, principal component, (+)–piperitenone oxide] and *M. spicata* var. *crispa* [2n = 48, principal component, (–)–carvone]. Piperitenone oxide has not been reported in the oil of *M. arvensis* of different geographical origins surveyed by Handa *et al* (5) which contains menthol and menthone as the principal constituents. The oil of *M. arvensis* L. obtained from Europe and cultivated in Japan is levorotatory and contains 3-octanone as the principal component according to Shimizu *et al.* (14)

TABLE 1.—*Oil yield and physical constants of oil of Philippine mint and oils of Japanese mints.*

	Philippine mint			Japanese mints	
	Young vegetative tops	Mature vegetative parts	Flowering tops	*M. rotundifolia* (10)	*M. spicata* (2n = 24) (12)
Oil yield (per cent air-dry weight)	1.50–2.39	0.64–1.08	1.55–3.76	0.06–0.19[a]	0.31, 0.16[b]
Maximum absorption (in absolute methanol)	0.453 at 268 mμ	0.216 at 260 mμ	0.642 at 260 mμ	0.260–0.402[c] at 260 mμ	0.38, 0.20[c] at 260 mμ
Optical rotation (°)	+141.01[c] at 29°C	+131.67[d] at 29° C	+124.50[d] at 29° C	+82.3–+155.15 at 15° C	+39.04, +68.25 at 10°C
n^{28}_{D}	1.4452	1.4561	1.4611	1.5000–1.5063 at 27° C	1.4855, 1.4625 at 10°C
Specific gravity	1.0247 at 30°C	1.0201 at 30.5°C	1.0241 at 30.5°C		

[a] Percentage w/w on a fresh-weight basis
[b] Percentage basis not indicated.
[c] Ten μg/ml in methanol or ethanol
[d] Two per cent v/v solution in absolute methanol

Stolons from the Philippines grown at the farms of the A. M. Todd Company in Michigan matured and bore flowers in terminal spikes and the flowering Philippine mint resembled most, in fact was indistinguishable from, *M. cordifolia* Opiz. This species is listed by Clapham *et al* (3) as a hybrid between *M. rotundifolia* and *M. spicata*. Stolons of authentic *M. cordifolia* were obtained from the same company and grown at the University of the Philippines. The vegetative habit of the plant is the same as that of Philippine mint or yerba buena under greenhouse conditions (Plate 2).[1]

SUMMARY AND CONCLUSIONS

Physiological and chemical studies of Philippine mint or yerba buena indicate that it is not *Mentha arvensis* L. as it was previously thought to be. The identification of the different members of the genus *Mentha* is not easy nor simple since each species has more than one chromosome number and easily produces fertile hybrids with other strains. Present evidences indicate that Philippine mint should be more properly called *M. cordifolia* Opiz.

ACKNOWLEDGMENT

These studies were supported by the University of the Philippines Natural Sciences Research Council and the National Research Council of the Philippines. The cooperation and help of Dr. Merritt J. Murray, geneticist of the A. M. Todd Company, Kalamazoo, Michigan; Dr. Wallace R. Ernst, associate curator, Department of Botany, U. S. National Museum, Smithsonian Institution, Washington, D. C.; and Dr. Lorin I. Nevling, Jr., Supervisor of the Herbaria, Harvard University, Cambridge, Massachusetts are gratefully acknowledged. Analyses of the oil were conducted at the Takasago Perfumery Company, Tokyo, through the kindness of Drs. A. Komatsu and S. Muraki. The author acknowledges with thanks the cooperation of the chairmen of the Department of Botany and of the Department of Physics of the College of Arts and Sciences, University of the Philippines, for the use of their facilities and the technical assistance of Miss Ma. Vicenta T. Cuevas, Miss Nerissa S. Vargas, and Mrs. Florian Magno-Orejana.

[1] Four months later, the two plants flowered under interrupted nights, both developing identical terminal spikes.

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ILLUSTRATIONS

PLATE 1

FIG. 1. Philippine mint grown under three different photoperiods. The plant flowered under long days and under short days with interrupted nights.

2. Flowering top of Philippine mint grown in the field under natural nights interrupted with 2 hours of light of relatively low intensity.

PLATE 2

FIG. 1. Philippine mint or yerba buena (left) and authentic *Mentha cordifolia* Opiz (right) grown together in the greenhouse.

1

2

PLATE 1.

PLATE 2.

THE CAT FLEA HITHERTO UNKNOWN TO SUSTAIN THE LARVA OF DIPYLIDIUM CANINUM (LINNAEUS, 1758) FROM THE PHILIPPINES

By L. M. Yutuc

ONE TEXT FIGURE

In a revision of the genus *Dipylidium* Leuckart, Rodriguez Lopez-Neyra (1929) summarized the previous works on the intermediate hosts of *Dipylidium caninum*, a cosmopolitan tapeworm of the dog and cat and occasionally human as well as wild feline and canine subjects. Initial experiments were carried by Melnikow in 1869, followed by Villot in 1883, Grassi and Sonsino in 1888. Detailed studies were those of Grassi and Rovelli in 1889-1892, and Joyeux in 1916–1920. The intermediate hosts incriminated were the dog louse, *Trichodectes canis*, the dog flea, *Ctenocephalides canis*, and the human flea, *Pulex irritans*. Verand (1938) citing the experimental findings of Chen in 1934, stated that the cat flea, *Ctenocephalides felis* is capable of sustaining the larval states of *Dipylidium caninum*. In 1937 Zimmermann confirmed previous findings that *Ctenocephalides canis* and *Ctenocephalides felis* are intermediate host of *Dipylidium caninum* but not *Trichodectes canis* [Stewart (1939)]. As far as available literature disclosed the cat flea has never been noted in nature as harboring the immature stages of *Dipylidium caninum* [Tongson and de Vera (1967)] in the Philippines, hence the present report. The materials hereto described were covered partially by an abstract entitled, "Juvenile Worms of Cat Flea, *Ctenocephalides felis* from the Philippines," presented in a meeting of the American Society of Parasitologists, held in 1967, Tucson, Arizona.

In this report, 1,023 cat fleas *Ctenocephalides felis felis* were collected from dogs and cats in 1966 and dissected alive at various intervals in one to two drops of saline solution and examined microscopically. The results of flea dissection are presented in Table 1. Of this number of fleas, 768 were females and 255 males.

TABLE 1. *The incidence of D. caninum immature stages in cat flea in the Philippines.*

C. felis	Number examined	Number positive	Per cent positive	Number of worms recovered	Average number of worms per infected flea
Male	255	7	2.7	32	4.6
Female	768	18	2.3	115	6.4
Both	1 023	25	2.4	147	5.9

Twenty-five fleas or 2.4 per cent of total were found harboring immature stages of the tapeworm. Of these 25 infected fleas, 18 were females and 7 males, resulting in infection rates of 2.3 per cent and 2.7 per cent, respectively. A total of 147 worms was recovered from these fleas, that is, 115 from the females and 32 from the males, giving an average of 6.4 worms per infected female flea and 4.6 worms per infected male flea. The difference in worm burden may be attributed to the larger size of the female.

Morphological studies revealed roughly three stages; namely, 81 procercoids, 55 cysticercoids, and 11 evaginated cysticercoids showing a preponderance of first stage larvae. The heaviest infection was encountered in a female flea from a kitten, with 40 cysticercoids and four evaginated cysticercoids. Next in rank and in diminishing order were from a male flea and the other female both of cat origin with 24 and 17 procercoids respectively. With the exception of a female flea of a dog with 13 procercoids, the rest sustained seven to one larval stages, the latter predominating.

At the time these positive infected fleas were found, the dogs and the cats were passing almost daily segments of *Dipylidium caninum*. Moreover, one dog and a cat died. On autopsy the dog yielded 30 and the cat one tapeworm indistinguishable from the morphologic features of *Dipylidium caninum*.

Larval stages. The procercoid is an elongated white opaque body; measures in a preserved and stained condition 0.25 by 0.13 mm; range 0.18–0.40 by 0.12–0.13 mm Attacked to its posterior end is a tail-like structure, the cercomer, an easily detached organella. In it are hooks. It

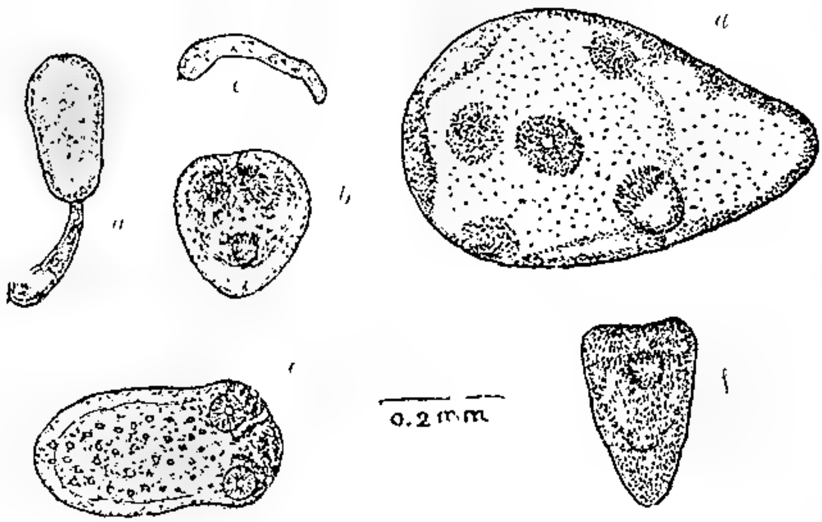

FIG. 1. Larvae of *Dipylidium caninum* in the flea: *a*, procercoid; *b*, cysticercoid; *c*, cercomer; *d*, atypical cysticercoid; *e*, ... and *f*, evaginated cysticercoid.

is claimed that these are the hooks found embedded in the eggs of the tapeworm. In the anterior pole is a slight depression carrying in it minute spines (Fig. 1*a*).

The typical form of the cysticercoid is pyriform provided with a nest of three rows of rose-thorn shaped hooks generally located close or slightly posterior to the center of the embryo supported by a cup-shaped body, the two constituting the rostellum. Anterior to this organ are four suckers quite visible, hugging close to a depressed anterior pole of the larva. In some specimens the excretory canal can be noted immediately behind the rostellum extending on both sides and running anteriorly up close to the suckers (Fig. 1*b*). The cysticercoid measures 0.35 by 0.31 mm; range 0.43 to 0.31 by 0.32 to 0.29 mm. The cercomer has been observed in stained preparation but always detached from the embryo (Fig. 1*c*).

A typical form, quite large (0.7 by 0.4 mm) was occasionally found especially in fleas collected from cats. The suckers were haphazardly distributed but still anterior to the rostellum, the latter being located near the wall of the

cysticercoid and more posteriorly than the usual site of this structure in typical embryo. The anterior depression is entirely missing. Whether this condition is due to the state of relaxation at the time the larva was preserved and stained is subject of further inquiry (Fig. 1*d*).

The evaginated cysticercoid [Whitlock (1960)], a more or less elongated body measures 0.59 long by 0.38 mm wide with a range of 0.64 to 0.5 by 0.24 to 0.25 mm. The suckers and the rostellum are invariably situated close to the anterior pole. Sometimes the rostellum is more anterior than the suckers, in other vice versa. The excretory canal is quite conspicuous coursing from posterior to an anterior direction (Fig. 1*e* and 1*f*). Common to all stages are the calcarious corpuscles and the active movements by relaxation and contraction of the embryo in fresh preparation. Verand claim this stage to be laterally compressed cysticercoid.

For comparative purpose measurements of the cysticercoid and evaginated cysticercoid encountered in a female flea heavily infected with 44 larvæ were made. In this particular instance the cysticercoid measures 0.22 by 0.2 mm; range 0.27 by 0.24 to 0.17 by 0.18 mm; the evaginated cysticercoid 0.29 by 0.2, range 0.32 to 0.27 by 0.2 to 0.18 mm indicating a much reduced size in both embryonal stages due perhaps to crowding of the hemal fluid of the flea quite similar to the state as that observed with heavy infection of rats with *Hymenolepis diminuta* when larger number of tapeworms per host induce a smaller individual worm size and vice versa [Roberts (1961)]. Since heavy infection of the larval flea with the ova of *Dipylidium caninum* invariably results in the death of the host, this case mentioned above appears exceptional.

Although the above finding agrees with the results of investigators abroad particularly Chen, it did not support the recent research note of Tongson and de Vera who claimed the absence of cysticercoids in 1,375 fleas collected from Philippine dogs despite the greater number employed than the one hereto presented. This state of affairs may be ascribed to different techniques employed and to some extent to environmental conditions operating at the time the observations were performed.

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CONTRIBUTIONS TO A REVIEW OF PHILIPPINE SNAKES, XI

THE SNAKES OF THE GENUS BOIGA

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TWO TEXT FIGURES

The rear-fanged arboreal snakes of the genus *Boiga* are distributed from Australia, on the east, to Afghanistan and western Pakistan, on the west, and following a hiatus of major proportions, central Africa Though occasionally found in semiarid or arid environments, such as the Dasht-i-Margo of Afghanistan [Leviton (1959) 457], members of the genus favor more tropical conditions.

Five species of *Boiga* are found in the Philippines. One, *B. philippina,* is known from a single specimen taken on northern Luzon and is doubtfully distinct from its congener, *B. angulata*. The four remaining species are widely distributed, one or more being found on each of the larger islands.

The Philippine species of *Boiga* are clearly related to Indonesian forms, indeed for the most part scarcely more than subspecifically distinct from populations inhabiting Borneo. Inadequate samples prevent a more detailed assessment of the taxonomic status of the several nominal species currently recognized.

TERMINOLOGY

Standard length: Distance from tip of snout to anal opening. Measurements given under "Diagnosis" are for the largest specimen of each sex studied, both standard and tail lengths being given.

Tail length: Distance from anal opening to tip of undamaged tail.

* : [asterisk] following locality listed under "Range" indicates sites from which specimens were examined.

23 (–3 [144–145]) 21: Dorsal scale reduction formula indicates a reduction from 23 to 21 longitudinal rows of scales

by loss of the third row on each side between ventrals 144 and 145 in the series of specimens examined. Caudodorsal scale reductions determined by level of subcaudal shield opposite which reduction takes place.

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Genus BOIGA Fitzinger

Boiga FITZINGER (1826) 31 (type species *Coluber irregularis* Merrem, by original designation).

Dipsadomorphus FITZINGER (1843) 27 (type species *Dipsas trigonata* Schlegel, by original designation).

Gonyodipsas FITZINGER (1843) 27 (type species *Dipsas irregularis* Merrem [of Schlegel], by original designation)

Cephalophis FITZINGER (1843) 27 (type species *Dipsas dendrophila* Reinwardt in Boie (1827) by original designation).

Eudipsas FITZINGER (1843) 27 (type species *Dipsas cynodon* Cuvier, by original designation).

Macrocephalus FITZINGER (1843) 27 (type species *Dipsas drapiezii* Boie, by original designation).

Opetiodon DUMÉRIL (1853) 491 (type species *Dipsas cynodon* Cuvier [of Schlegel], by monotypy).

Triglyphodon DUMÉRIL (1853) 507 [type species *Coluber irregularis* Merrem, by subsequent designation by Stejneger, (1907)].

Toxicodryas HALLOWELL (1857) 60 (type species *Toxicodryas blandingii* Hallowel, by monotypy).

Pappophis MACLEAY (1877) 39 (type species *Pappophis laticeps* Macleay, by monotypy).

Liophallus COPE (1895) 427 (type species *Dipsas fusca* Gray [of Cope], by monotypy).

Definition.—Maxillary teeth 10 to 16, subequal except for the last two or three which are enlarged and grooved; palatine teeth often strongly enlarged: ectopterygoid more or less distinctly forked anteriorly, both branches articulate with maxilla; head distinct from neck; snout short, blunt; eye large, pupil vertical; body somewhat compressed; scales smooth, with apical pits, in 19 to 23 longitudinal rows, the vertebral series frequently enlarged; ventrals usually obtusely angulate laterally; tail moderate or long; subcaudals paired; hypapophyses present or absent on posterior vertebræ. [After Smith (1943) 344.]

Remarks.—The snakes of this genus are usually found in arboreal situations, in among the branches of bushes and trees. They are characterized by possessing a short, blunt head which is very distinct from the neck, an elongate and compressed body, moderate to long tail, enlarged vertebral scales, and ventrals which angulate laterally. The very large eyes and vertical pupil suggest nocturnal habits. Smith [(1943) 346] states that these snakes lie coiled up on branches of trees, differing thereby from other arboreal snakes, such as *Dendrelaphis* and *Ahaetulla*, which lie extended upon branches.

In defining the genus *Boiga* Smith [(1943) 244] stated that "Hypapophyses [are] present on the posterior dorsal vertebræ in all the Asiatic species." Brongersma [(1938) 241–242] examined specimens of *Boiga cynodon, B. jaspidea, B. multimaculata* and *B. irregularis*. He failed to find hypapophyses present in the first three species. In *B. irregularis* their presence was variable; five of seven specimens possessed hypapophyses; one specimen had only a low keel; and one specimen lacked hypapophyses altogether. I am able to confirm Brongersma's observations to the extent that the presence or absence of well defined hypapophyses on the posterior dorsal vertebræ is variable. I have examined specimens of *B. cynodon, B. dendrophila, B. irregularis, B. karclini*, and *B. angulata* and find that a ridge is usually present along the base of the centrum, and that in some individuals the posterior portion of this ridge projects downward slightly, forming a small hooklike projection. In only one specimen of *B. cynodon* was the ridge completely lacking.

Four nominal species, one polytypic with two nominal subspecies, were recognized by Taylor in 1922. A year later

Taylor added a fifth nominal species of *Boiga* to the Philippine fauna. Of these five, *B. philippina*, known from the type only, cannot reasonably be differentiated from *B. angulata*, a species widely distributed throughout the Philippines; though it probably should be referred to the synonymy of *B. angulata*, it is retained here since I have not seen Peters' type. Both *B. angulata* and *B. schultzei*, the latter described by Taylor in 1923 from Palawan, are close to *B. drapiezi*, a widely distributed and evidently variable snake known from Borneo and elsewhere in western Indonesia. It is my guess that two species currently parade under the name *B. drapiezi* and that one of these is more closely allied to *B angulata;* lack of adequate samples to evaluate pattern variation leads me to take a conservative approach and retain the arrangement of earlier authors. *Boiga dendrophila*, the brilliantly colored mangrove snake of the Indo-Malayan region, is represented in the Philippines by three distinct populations. Brongersma (1934) reviewed this species complex; I have accepted his arrangement because our data are in complete agreement. Lastly, *Boiga cynodon*, another species widely distributed throughout the Indo-Malayan region, is treated as a monotypic species in the Philippines. Taylor [(1923) 554] has pointed out that there is extensive variation in color and markings, and it is difficult to find two specimens marked alike. Further, neither differences in color pattern nor other known variations can be associated with geographic distribution.

Key to the Philippine species and subspecies of Boiga

1a. Body color very dark brown or black with a series of yellow or white crossbars; scales in 21 longitudinal rows at midbody.

2a. Yellow or white crossbars less than two scale rows wide, not expanded laterally.

3a. Interspaces between narrow light crossbars black (Palawan Archipelago) . . . . *B. dendrophila multicincta*

3b. Interspaces between narrow light crossbars, which are edged with black, bluish gray to bluish brown (Luzon and Polillo). *B. dendrophila divergens*

2b. Yellow or white crossbars two or more scale rows wide, which become wider on sides (Mindanao and Samar). *B. dendrophila latifasciata*

1b Body color light brown, with or without darker brown crossbars; **scale rows variable.**

4a. Scales in 23 longitudinal rows at midbody; 9 upper labials; 3 anterior temporals . . . . . *B. cynodon*

4b. Scales in 19 longitudinal rows at midbody; 8 upper labials; 2 anterior temporals.

5a. One preocular; ventrals more than 250.

6a. Posterior chin shields smaller than anterior shields; loreal present; 39 to 42 brown crossbars on body which widen laterally . . . . . *B. angulata*

6b Posterior chin shields as large as anterior shields; loreal present or absent; about 70 very narrow dark brown crossbars on body which do not widen laterally . *B. schultzei*

5b. Two preoculars; ventrals less than 250 . *B. philippina*

BOIGA ANGULATA (Peters).

Dipsas (Dipsadomorphus) angulata PETERS (1861) 688 (type loc. Leyte; type in Berlin Museum; original description); BOETTGER, (1886) 113 (listed), (1892b) xlix (Catanduanes; listed).

Dipsadomorphus angulatus, BOULENGER (1896) 75 (description after Peters); BOETTGER (1898) 93 (Catanduanes; listed).

Boiga angulata, GRIFFIN (1910) 213 (Polillo; food), (1911) 263 (distribution compiled; listed in key); TAYLOR (1917) 366 (Negros [Mt. Marapara]; listed), (1922a) 204, pl. 26, figs. 1–3, pl. 27 (Catanduanes, Leyte, Negros, Luzon [Los Baños], Polillo, synonymy, description, counts and measurements of material examined), (1922c) 139 (Luzon [Mt. Makiling]; listed), (1923) 553 (comparison with *B. schultzei*); LEVITON (1963) 383, 389, 390, 398, 402 (listed from Catanduanes, Leyte, Luzon, Mindanao, Negros, and Polillo).

Dipsas (Eudipsas) quiraonis STEINDACHNER (1867) 75, pl. 3, figs. 9–10 (type loc: Philippine Islands; types in Vienna Museum; original description); MÜLLER (1883) 289 (Mindanao; listed); FISCHER (1885) 81 (Mindanao; listed); BOETTGER (1886) 113 (distribution compiled; listed).

Range.—CATANDUANES: (without exact locality *). LEYTE: (without exact locality). LUZON: Laguna Province (Los Baños *; Mt. Makiling *). MINDANAO: Bukidnon Province (Del Monte Plantation *); Cotabato Province (Tatayan * [= ? Talayan]); Davao Province (Caburan *); Lanao Province (Lake Lanao *). NEGROS: (Pagyabunan *; Mt. Marapara [cannot be located; may be Mt. Malapanta). POLILLO: (without exact locality *).

Material examined (9).—CATANDUANES: without exact locality (CAS 15315. LUZON: Laguna Province: Los Baños (MCZ 25792); Mt. Makiling (CAS 61305). MINDANAO: Bukidnon Province: Del Monte Plantation (SU 12360); Cota-

bato Province: Tatayan (FMNH 122026); Davao Province: Malita, Caburan (FMNH 53468); Lake Lanao (CAS 15316). NEGROS: Bais, Pagyabunan (FMNH 61620). POLILLO: without exact locality (CAS 62421).

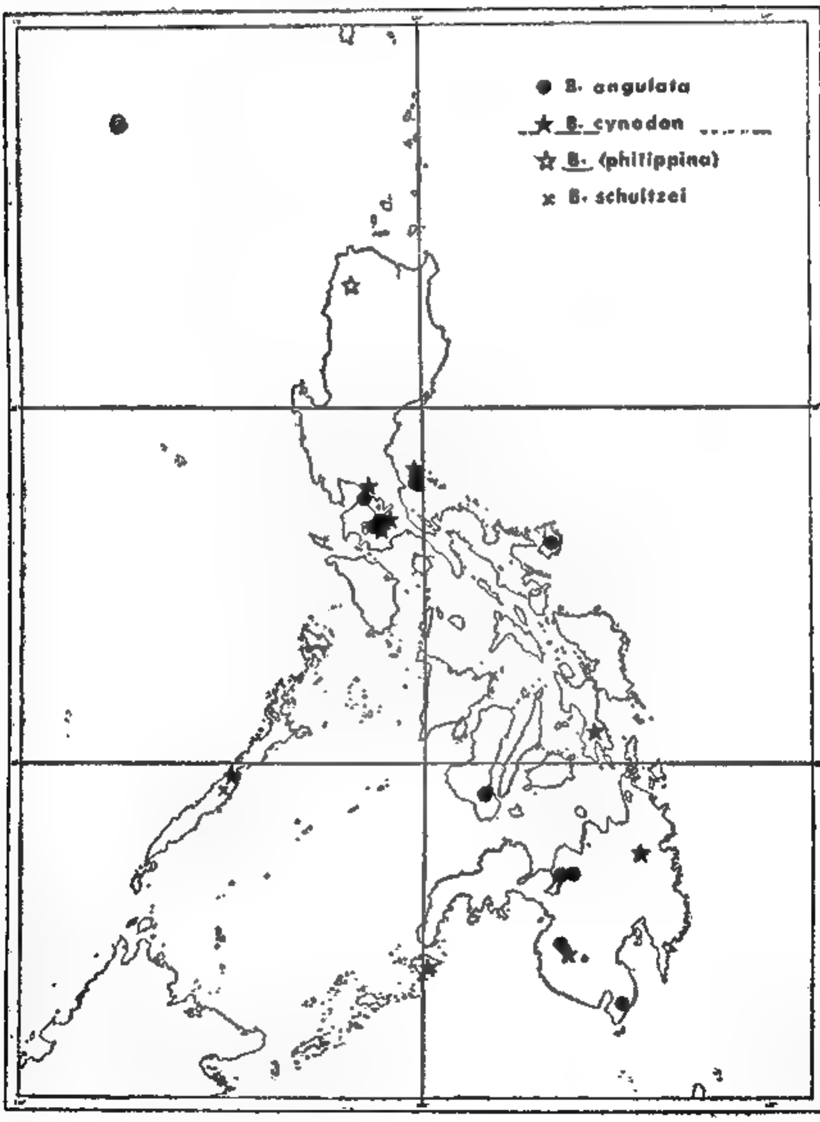

FIG. 1. Distribution of *Boiga angulata*, *B. cynodon*, *B. philippina*, and *B. schultzei* in the Philippines.

Taxanomic notes.—This species is close to *Boiga drapiezi* from which it appears to differ in possessing a somewhat larger loreal shield and, though this is by no means constant and a complete series of intermediates is known, in the proportionally smaller size of the posterior chin shields compared to the anterior pair. These are not very significant differences and it seems likely that examination of adequate samples of both forms will lead to the union of the two taxa. There appears to be two color pattern varieties of *B. drapiezi*, one of which is almost identical to that of *B. angulata;* the pattern is formed of a series of light brown saddle-shaped blotches bordered by darker bars; the other variety of *B. drapiezi* has light and dark cross bands which do not expand middorsally and a distinct middorsal light stripe extending onto the tail.

I have examined 13 specimens of *Boiga drapiezi* from Sarawak, Borneo. These 13 specimens may be divided into two groups, one having a series of saddle-shaped light brown blotches separated by dark brown crossbars which widen on the sides on the dorsum, the other a series of dark and light crossbars of uniform width and a solid middorsal stripe. Though the samples are too small for adequate statistical analysis of the meristic data, a comparison of ventral counts between the males, which are in the majority, is instructive. Six males having crossbars and a middorsal white stripe average 278.3 $\pm$ SE 3.31 ventrals, while four males having saddle-shape blotches and lacking the white stripe average 263.3 $\pm$ SE 5.23 ventrals. The difference in average ventral counts is not immediately striking until a comparison is made with counts obtained from a series of Philippine *Boiga*, nominally referred to *B. angulata*, which have saddle-shaped blotches, lack a white middorsal stripe, and otherwise look like their Sarawak counterparts. Five males from scattered localities in the Philippines average 262.6 ventrals, a remarkable coincidence if we are to believe that in Sarawak both color patterns are to be referred to *B. drapiezi*. A further observation is also instructive. Sexual dimorphism in ventral counts is only weakly developed, so it is possible to combine data for males and females. The cross-barred Sarawak sample of males and females includes eight individuals; there is an average of 276.4 $\pm$SE 3.75 ventrals (range 256 to 287). The blotched Sarawak sample (males and females) of five

specimens average 265.8 ± SE 4.76 ventrals. The Philippine sample (males and females) of *B. angulata* consists of nine specimens. These average 263.8 ± SE 1.14 ventrals, again a remarkable concurrence with the blotched Bornean sample. I suspect that in fact two species have for a long time paraded under the name of *B. drapiezi*, and that one of them is more widely distributed than the other, being found throughout eastern and northern Philippines as well as on Borneo.

Boiga drapiezi was described by F. Boie in 1827, evidently based on a series of specimens said to have come from Java. His description is not adequate to determine which of the two forms he had before him, nor are the data published later by Boulenger (1896) or de Rooj (1917) especially helpful. At the moment I must deal with what I know and therefore take the conservative position retaining the taxa in their present dubious states. Though both Boulenger [(1896) 74] and de Rooj (1917) point out that two distinct color patterns are recognizable in *B. drapiezi*, they make no attempt to correlate these patterns with other characters, nor do they present any data enabling others to do so. Consequently, examination of a more substantial series of these animals from western Indonesia must be made.

Boiga philippina (Peters) appears to be very similar to *B. angulata*. According to Peters the anterior palatine teeth are very greatly enlarged, there are two preoculars, and there are 240 ventrals. Inasmuch as the species is known from the type specimen alone, it has not been possible to compare it with *B. angulata*. I suspect that the two nominal species will be found to be quite similar, however.

Diagnosis.—Scales in 19 longitudinal rows at midbody; loreal present; preocular reaches upper surface of head; posterior chin shields tend to be smaller than anterior pair; dark brown crossbars on dorsum expand on sides and extend onto ventrals, lighter blotches saddle-shaped; a series of discrete spots present along lateral edge of ventrals, usually not forming a continuous line. Standard length: ♂ 1055; ♀ 916; tail length: ♂ 367, ♀ 312.

Desciptive notes.—Maxillary teeth 14; anterior palatine teeth only moderately enlarged; 1 preocular which reaches upper surface of head; well developed loreal present; 2 postoculars; temporals 2 + 2 or 2 + 3; 8 upper labials, third, fourth and

fifth bordering orbit; 10 lower labials, the first five in contact with anterior chin shields; posterior chin shields shorter and narrower than anterior pair; ventrals 254 to 267; subcaudals 126 to 153; anal plate single.

Hemipenes extend to end of eighth subcaudal plate, unforked; sulcus spermaticus unforked; spinose throughout with large spines present in proximal half followed by an abrupt reduction with smaller spines in the distal half; a few shallow calyces present at distal end.

Color (in alcohol) above light brown saddle-shaped blotches with a series of 39 to 42 dim, darker brown crossbars and with numerous minute dark brown spots; dark stripe present or absent along lateral edges of ventrals.

Sexual dimorphism.—Other than a difference in the number of subcaudals, which are more numerous in males than in females, the sexes do not seem to differ. The absence of interisland variation permits grouping of data for all of the island samples. These are summarized in Table 1.

TABLE 1.—*Summary of variation between sexes in Boiga angulata.*

	Male			Female		
Character	N	Mean	Range	N	Mean	Range
Ventrals	5	262.6	259–267	4	265.3	261–270
Subcaudals	5	146.0	138–153	4	141.75	134–150
Body blotches	4	40.0	39–42	2	42.0	42
Tail length/standard length	6	0.348	0.331–0.380	2	0.347	0.341–0.352

Ecological notes.—Of the three Field Museum specimens examined, two were collected in the forest at Caburan, at an altitude of 36 meters, and one on the forest floor at Tatayan [= ? Talayan] [altitude 2,500 meters].

BOIGA CYNODON (H. Boie).

Dipsas cynodon H. BOIE (1826) 238 (*nomen nudum*); in F. BOIE (1827) column 549 (type loc.: Sumatra; type in Leiden Museum; original description); GÜNTHER (1864) 308 (description; listed as occurring in the Philippines); JAN (1871) Livr. 38, pl. 6, figs. 1 2; GÜNTHER (1879) 78 (Mindanao; listed); BOETTGER (1895) 4, 5 (Calamianes Ids.; also stated to occur in Mindanao and Luzon; scutellation, counts).

Dipsadomorphus cynodon, BOULENGER (1896) 78 (synonymy, description, counts of material examined); BOETTGER (1898) 94 (Culion, Luzon [Manila]; listed).

Boiga cynodon, GRIFFIN (1909) 599 (Mindanao, Palawan [Iwahig]; listed), (1910) 213 (Polillo; listed), (1911) 264 (distribution compiled; listed in key); TAYLOR (1922a) 206 (Culion, Luzon [Los Banos], Mindanao [Bunawan], Polillo, Palawan [Iwahig], Leyte; synonymy, description, counts and measurements of material examined), (1922c) 139 (Luzon [Mt. Makiling]; ecological note), (1923) 553 (Basilan [Abung-Abung], Luzon [Mt. Makiling], "? Palawan (or Balabac)," Polillo; scutellation, measurements and counts); LEVITON (1963) 379–385, 389, 390, 393, 400, 403 (listed from Basilan, Culion, Leyte, Luzon, Mindanao, Palawan, and Polillo).

Range.—(Philippine localities only.) BASILAN: Abung-Abung *. CULION: without exact locality. LEYTE: without exact locality. LUZON: Laguna Province (Los Baños *; Mt. Makiling); Rizal Province (Manila). MINDANAO: Agusan Province (Bunawan *); Cotabato Province (Saub *; Tatayan * [= ? Talayan]). PALAWAN: * Iwahig *, Puerto Princesa *. POLILLO: without exact locality.

Material examined (13).—BASILAN: Abung-Abung (CAS 603348); without exact locality (USNM 37432). LUZON: Laguna Province: Los Baños (MCZ 25796); Mt. Makiling (CAS 61301–61303). MINDANAO: Agusan Province: Bunawan (CM 2144); Cotabato Province: Saub (MCZ 25794); Tatayan [= ? Talayan] (MCZ 25795); without exact locality: (CAS 44140). PALAWAN: Puerto Princesa (CAS 15811); without exact locality (MCZ 25795). POLILLO: without exact locality (CAS 62422).

Taxonomic notes.—This distinctive species is widely distributed throughout southeastern Asia where it is said to inhabit the plains and hill country at low altitudes [Smith (1943) 358]. There has been no attempt to distinguish geographical populations, except by Boulenger [(1896) 79], and based upon the present study no division is called for. There is a remarkable homogeneity among samples of this species, and except for slight intra-population variation in color pattern, the species throughout its entire range is monomorphic.

Diagnosis.—Scales in 23 longitudinal rows at midbody; vertebral scales enlarged; subcaudals 122 to 157. Standard length: ♂ 1,250, ♀ 1,300; tail length: ♂ 397, ♀ 383.

Descriptive notes.—Maxillary teeth 11 to 14; anterior palatine teeth greatly enlarged; 1 preocular, frequently in contact

with frontal; 2 postoculars; temporals 2 + 3 or 3 + 3; 9 upper labials, rarely 8 or 10, fourth, fifth and sixth shields bordering orbit, rarely third through fifth or fourth through seventh; posterior chin shields longer than anterior pair; dorsal scales reduce 23 (-3[144—145]) 21 (vertebral + paravertebral [146-148]) 19 (vertebral + paravertebral [156]) 17 (vertebral + paravertebral [176]) 15; caudodorsal scales reduce 6 (2 + 3 [49–50]) 4; ventrals 250–282; subcaudals 120–157; anal plate single.

Hemipenes extending between eleventh and seventeenth subcaudal plates, unforked; sulcus spermaticus unforked; spines absent; proximal half to two-thirds with a series of smooth longitudinal folds; distal third to half with a few transverse plicæ and large, shallow calyces.

Color (in alcohol) light brown above, the dorsum usually crossed by a series of from 33 to 49 darker brown, black-edged crossbars of three to five scale rows in width, which narrow slightly on sides; tail crossed by 23 to 37 crossbars which are separated by very narrow lighter interspaces; below light brown thickly powdered with darker punctuations except under tail where dark color predominates and there are small light spots; a brown stripe extends from eye to angle of jaw; nape frequently with two narrow parallel brown stripes, or with a single dark brown spot between parietals; in some individuals all markings are absent from body.

Sexual dimorphism.—Because of the small size of the available sample it has not been possible to demonstrate whether the sexes differ in any character. There is a suggestion (Table 2) that males may have more ventral shields than females, but more adequate sampling is needed before a decision can be reached.

TABLE 2.—*Summary of variation between sexes in material examined of Boiga cynodon.*

	Male			Female		
Character	N	Mean	Range	N	Mean	Range
Ventrals	3	279.0	276–281	7	271.4	259–279
Subcaudals	3	139.3	136–150	8	140.3	132–157
Tail length/standard length	2	0.302	0.286–0.317	7	0.327	0.295–0.382
Crossbars on body	1	34		5	38.8	33–49
Crossbars on tail	1	23	----	5	27.8	21–37

Inter-island variation.—There is no clear evidence to indicate that known variations can be associated with geographic distribution. The absence of distinctive differences between sexes permits grouping the data for all of the island samples as has been done in Table 2.

Ecological notes.—According to Smith [(1934) 358] this snake inhabits the plains and hill country at low altitudes. All the specimens I have seen were taken at low altitudes. It is evident from body form that the snake is arboreal, but nowhere can I find a statement about the habitat in which specimens have been collected. The principal food items, which consist of birds and eggs, would indicate an arboreal habitat. The breeding habitats are unknown, but it is presumed that the snake is oviparous as are other members of the genus

BOIGA DENDROPHILA (F. Boie).

Dipsas dendrophila F. BOIE (1827) column 549.

Taxonomic notes. Three subspecies of *Boiga dendrophila* have been recognized in the Philippines [Taylor (1922a) 196–203; BRONGERSMA (1931) 201, 261–219]. I have seen a total of 25 specimens representing these three forms and find they are readily distinguishable. The earlier results of Taylor and Brongersma are, therefore, accepted and have been followed here.

Diagnosis.—Scales in 21 (rarely 23) longitudinal rows at midbody; usually eight upper labials; preocular reaches dorsum of head; body color black or dark slate, crossed by yellow or white bars which may be narrow or broad but not as broad as black interspaces.

Description.—(Composite description of Philippine populations only.) Head very wide across temporal-parietal region, tapering anteriorly; snout short, blunt, 1.1 to 1.6 times longer than horizontal diameter of eye; rostral about as broad as deep, barely visible from above; internasals not as deep as prefrontals; frontal slightly longer than broad, 1.1 to 1.6 times as wide as supraocular, shorter than parietals; nasal large, divided with large nostril; small loreal present, occasionally entering eye beneath preocular; 1 preocular, reaching dorsum of head; two postoculars; temporals usually 2 + 3; 8 upper labials, rarely 9, third, fourth and fifth bordering orbit; 10 to 11 lower labials, first pair in contact behind mental; first four shields in contact with anterior chin shields; posterior chin

shields as long as anterior pair, but somewhat narrower; scales in 21 longitudinal rows at midbody; ventrals 209 to 233; subcaudals 90 to 117; anal plate single.

Inter-island variations.—The principal variation among island populations is in color pattern. This includes both size of light crossbars and their number.

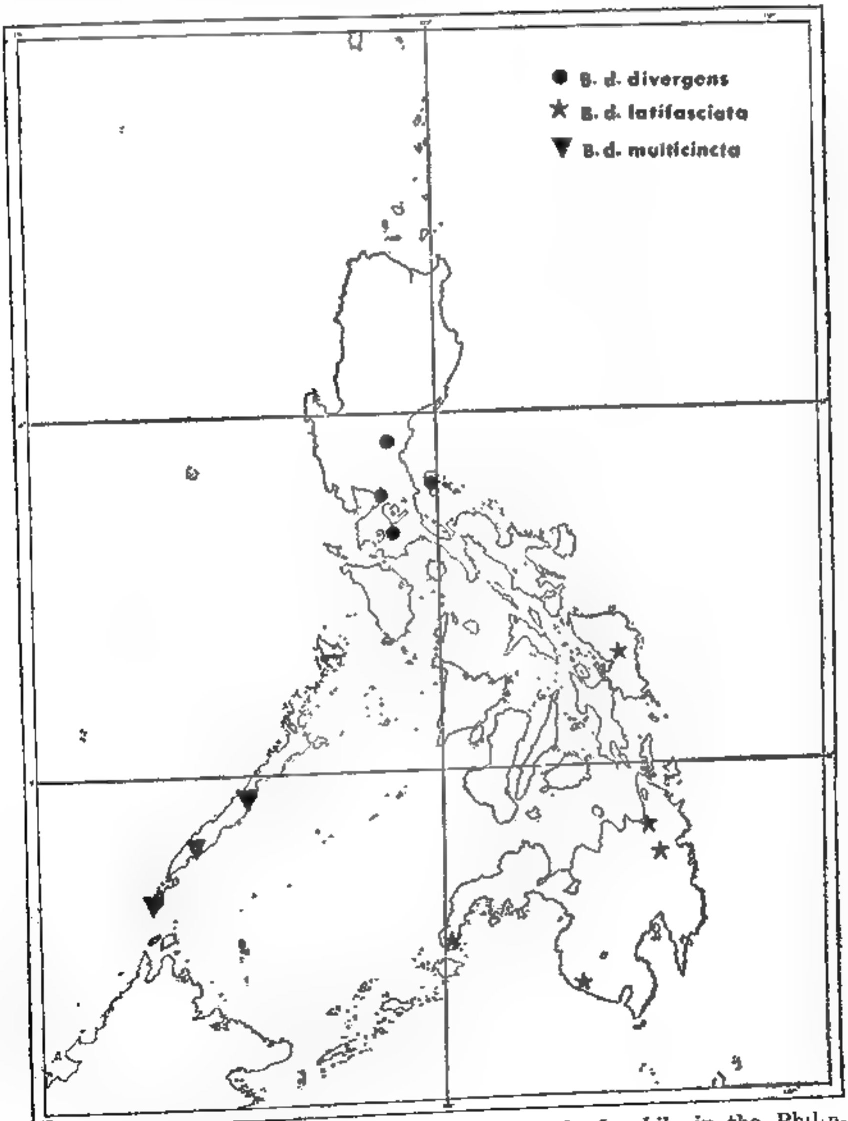

FIG. 2. Distribution of subspecies of *Boiga dendrophila* in the Philippines.

Specimens from Palawan and Balabac islands closely resemble the Bornean populations of *B. d. annectens*. The body is black; it is crossed by a series of narrow yellowish bars, each one scale row wide; these frequently fuse with adjacent bars on the sides, or if not, then the bars extend on to the lateral edge of the ventral shields. In specimens from Palawan and Balabac there are 58 to 80 [*fide* Brongersma (1934) 216] bars on the body (61 and 84 in specimens examined by me) and 21 to 29 bars on the tail (23 to 27 in specimens examined. Taking the total of bars on the body and tail, Brongersma notes that they vary from 85 to 111 (average 101.9 [97.6 in the specimens examined here]) or more bars than the average for Bornean populations.

The Mindanao-Samar sample differs from the above in having rather broad crossbars which at midbody are two to three scale rows wide [three or four ventrals wide according to Brongersma (1934) 218]. These crossbars are separated by black interspaces of at least equal width, although they are usually one or two scale rows wider, at least middorsally. The number of crossbars varies from 31 to 49 [Brongersma (1934) 218] on the body and 12 to 19 on the tail (36 to 46 and 12 to 18 respectively in the specimens examined by me).

Specimens from Luzon and Polillo islands more closely resemble the Bornean population than those from Mindanao and Samar islands. The ground color tends to be bluish-gray or slate; the crossbars are narrow, varying from one-half to one and one-half scale rows in width, are bordered by black, do not expand on the sides, and are separated by broad interspaces. There are from 55 to 79 yellowish crossbars on the body [76 to 79 according to Brongersma (1934) 217, 63 according to Taylor (1922a) 202] and 16 to 25 crossbars on the tail [25 to 27 according to Brongersma (1934) 218, 16 according to Taylor (1922a) 202] in specimens examined by me.

Comparison of ventral and subcaudal counts among samples from Luzon-Polillo, Mindanao-Samar, and Palawan-Balabac islands give evidence of inter-island variation in these counts which can be correlated with distribution and is not clinical in character. The Luzon-Polillo and Palawan-Balabac samples average about 10 ventrals more than the Mindanao-Samar sample. On the other hand, the Mindanao-Samar sample averages

about 100 subcaudals compared to 93 for the Luzon-Polillo sample and 110 for the sample from Palawan-Balabac.

It is evident from the above that recognition of three subspecifically distinct populations in the Philippines is fully justified.

BOIGA DENDROPHILA DIVERGENS Taylor.

Triglyphodon gemmicinctum DUMÉRIL, BIBRON, and DUMÉRIL (1854) 1091 (in part; locality given as Java but Brongersma believes description is that of Luzon population).

Dipsadomorphus dendrophilus multicinctus, BOULENGER (1896) 71 (in part; Philippines; counts of material examined); Boettger (1898) 93 (in part, Luzon [Dingalan; Nueva Ecija Prov.]; listed).

Boiga dendrophila, GRIFFIN (1909) 599 (in part; Luzon; listed), (1910) 214 (Polillo; listed), (1911) 263 (in part; Luzon [Rizal Prov.], Polillo; listed in key).

Boiga dendrophila divergens TAYLOR (1922a) 201 (type loc.: Mt. Makiling, Laguna Province, Luzon Island; type in Carnegie Museum; original description, color in life, counts and measurements of material examined), (1922b) 299 (Luzon [Mt. Makiling], Polillo; doubts Samar Island record given in earlier paper); (1922c) 139 (Luzon [Mt. Makiling]; specimens taken in branches of trees) Brongersma (1934) 317, pl. 1, figs. 5, 11, pl. 2, fig. 4 (distribution compiled; description of color pattern; summarizes counts); LEVITON (1963) 390, 403 (listed from Luzon and Polillo).

Range.—LUZON: Laguna Province (Mt. Makiling*); Nueva Ecija Province (Dingalan); Rizal Province (without exact locality*).

Material examined (3).—LUZON: Laguna Province: Mt. Makiling (CM 2143 [holotype], CAS 61304). POLILLO: without exact locality (CAS 62420).

Taxonomic notes.—In the original description Taylor noted a specimen in the collection of the Santo Tomas Museum which was said to have come from Samar Island. A specimen of *B.d. latifasciatus*, obtained by Dr. Moellendorf, was also said to come from that island. Although the sympatric occurrence of two subspecies of a single species is not without precedence, the present instance seems highly dubious. Zoogeographic considerations would suggest that *B. d. latifasciata* occur on Samar, and probably on Leyte Island too, but that the Luzon subspecies would not occur there. I believe it is reasonable to assume that there was some error in locality data accompanying the specimen in the Santo Tomas Museum.

Diagnosis.—Ground color slate or bluish-gray, with from 55 to 79 narrow, black-edged yellowish crossbars on the body; ventrals 219 to 228. Standard length: ♂ 624, ♀ 1095; tail length: ♂ 175, ♀ 250.

Descriptive notes.—Maxillary teeth 12; 1 preocular which reaches dorsum of head; 2 postoculars; temporals 2 + 2 or 2 + 3; 8 upper labials, third, fourth, and fifth bordering orbit; anterior chin shields as long as posterior pair; ventrals 219 to 228; subcaudals 80 to 95; anal plate single; scales in 21 longitudinal rows at midbody.

Color (in alcohol) brown to purplish brown above, the dorsum crossed by from 55 to 79 white, black-edged crossbars one-half to one and a half scale rows wide and separated by interspaces of four scale rows in width; several white spots on head scales including a line which originates on the supraocular and passes over the parietal, then to the angle of the jaw.

Sexual dimorphism.—As indicated by the data summarized in Table 3, there is marked dimorphism in the length of the tail, the number of subcaudals and the total number of ventral and subcaudal shields (*i.e.*, the total number of vertebræ). It is obvious that males have longer tails than females.

TABLE 3.—*Summary of variation between sexes of Boiga dendrophila divergens.* *

	Male			Female		
Character	N	Mean	Range	N	Mean	Range
Ventrals	7	222.3	219–225	1 **	228	----
Subcaudals	4	93.0	9[illegible]–95	1	80	----
Ventrals plus subcaudals	4	316.0	312–319	[illegible]	308	----
Tail length standard length	2	0.280	0.280–0.281	1	0.230	----

* Includes data from Taylor [(1922a) b and c] for specimens not otherwise examined in this study.

** Holotype.

A difference in the number of ventral shields is not very prominent; nevertheless the count for the single female is higher than the maximum number found among male specimens.

Inter-island variation.—Specimens of this subspecies have been taken on only two islands, Luzon and Polillo. Specimens from each island are very similar except for the number of

light crossbars on the body. The known number of crossbars on the dorsum for Luzon males ranges from 71 to 79 (mean 75.1). The single Polillo male specimen which I examined had only 55 crossbars. This represents a considerable reduction in the number of light bars on the body and indicates that the dark interspaces are considerably wider than among specimens from Luzon.

BOIGA DENDROPHILA LATIFASCIATA (Boulenger).

Triglyphodon dendrophilum var. B, DUMÉRIL, BIBRON, and DUMÉRIL (1854) 1090 (said to have come from Java; color description).

Dipsas dendrophila, GÜNTHER (1879) 78 (Mindanao [Butuan]; listed); FISCHER (1885) 81 (Mindanao; listed).

Boiga dendrophila, GRIFFIN (1909) 599 (in part; Mindanao; listed), (1911) 263 (in part; distribution compiled; listed in Key).

Dipsas (Triglyphodon) gemmicincta (nec DUMÉRIL, BIBRON, and DUMÉRIL) PETERS (1861) 688 (Mindanao; listed).

Dipsadomorphus dendrophilus latifasciatus BOULENGER (1896) 71 (type loc.: Mindanao; types in British Museum; specimens from Butuan, Agusan Province and Zamboanga Province, Mindanao; original description); BOETTGER (1898) 93 (Mindanao and Samar; listed).

Boiga dendrophila latifasciata, TAYLOR (1922a) 198 (Mindanao [Bunawan]; description, color in life, variation, counts and measurements of material examined); BRONGERSMA (1934) 218, pl. 1, figs. 6, 8, 10, pl. 2, figs. 10, 12 (Mindanao [Cotabato coast; Kapitan; Butuan; Zamboanga], Samar; description of color pattern scutellation; counts of material examined); LEVITON (1963) 393, 404 (listed from Mindanao and Samar).

Range.—MINDANAO: Agusan Province (Bunawan*; Butuan); Cotabato Province (Saub*); Zamboanga Province (Pantalan; Zamboanga City*). SAMAR: (without exact locality). PHILIPPINES: (without exact locality*).

Material examined (15).—MINDANAO: Agusan Province: Bunawan (CM 2138–2142); Cotabato Province: Saub (MCZ 25782–25789); Zamboanga Province: Pantalan (SU 12361); Zamboanga City (SU 8678). PHILIPPINES: without exact locality data (CAS 15313).

Diagnosis.—Ground color black with from 34 to 49 broad yellowish crossbars on body; ventrals 207 to 233. Standard length: ♂ 917, ♀ 926; tail length: ♂ 243, ♀ 266.

Descriptive notes.—Maxillary teeth 12 to 13; 1 preocular; 2 postoculars; temporals 2 + 2 or 2 + 3; 8 upper labials, rarely 7, third, fourth and fifth, rarely second, third and fourth shields bordering orbit; anterior and posterior chin shields

about equal; ventrals 207 to 226; subcaudals 93 to 102; anal plate single.

Color (in alcohol) dark brown to black above, the dorsum crossed by white or yellow crossbars which are two scale rows wide middorsally but which widen laterally to cover about four scale rows; yellowish below, each ventral edged with black on anterior portion of body, the black becoming more dense posteriorly where the black forms rings.

Sexual dimorphism.—Males average slightly fewer ventrals than females although the extremes for the sample of females encompass those for the males. There is no appreciable difference in the number of subcaudal shields, nor in the total of ventral plus subcaudal shields, the tail length/standard length ratio, or in the number of crossbars on the body and tail (Table 4). The absence of any clear dimorphism between sexes in this subspecies is rather interesting inasmuch as this differs from trends among other subspecies of *B. dendrophila.* This fact, combined with rather sharp differences in color pattern, suggests that *B. d. latifasciata* is not as closely related to the other two Philippine subspecies as they are to each other.

TABLE 4.—*Summary of variation between sexes in Boiga dendrophila latifasciata.* [a]

Character	Male			Female		
	N	Mean	Range	N	Mean	Range
Ventrals	10	214.7	209–222	9	213.2	207–226
Subcaudals	10	97.8	96–102	9	97.2	93–101
Ventrals plus subcaudals	7	307.8	300–314	9	304.3	[illegible]
Tail length/standard length	7	0.278	0.263–.301	6	[illegible]	[illegible]
Bars on body	5	42.3	34–47	6	42.5	[illegible]
Bars on tail	8	15.6	12–18	6	15.8	11–18

[a] Includes data from Taylor (1922a) and Brongersma (1934) for specimens not otherwise examined in this study.

Ecological notes.—According to Taylor [(1922a) 200] specimens were seen and collected only in low brush and trees and were never encountered on the ground.

Taylor found that one specimen obtained by him had just eaten a bat. I have found the remains of a skink (*?Mabuya*) and a lizard egg in the stomach of one individual.

Eggs were found in the oviducts of two specimens, three eggs in one individual, one in the other. Unfortunately the dates when these specimens were collected are not known.

BOIGA DENDROPHILA MULTICINCTA (Boulenger).

Dipsadomorphus dendrophilus multicinctus BOULENGER (1896) 71 (type loc.: restricted to Puerto Princesa, Palawan Island by Brongersma (1934); types in British Museum; original description [in part]).

Boiga dendrophila multicincta, TAYLOR (1922a) 200 (Balabac, Palawan [Iwahig]; description, measurements and counts of material examined); BRONGERSMA (1934) 216 (Palawan [Puerto Princesa]; description, counts of specimens examined); LEVITON (1963) 378, 400 (listed from Balabac and Polillo).

Boiga dendrophila, GRIFFIN (1910) 599 (in part; Palawan [Iwahig]; listed), (1911) 263 (in part; Palawan; listed in key).

Range.—BALABAC: (without exact locality*). PALAWAN: (Brooke's Point*; Iwahig*; Puerto Princesa*).

Material examined (8).—PALAWAN: Brooke's Point (MCZ 25790); Iwahig (CAS 62175); Puerto Princesa (CAS 15802). BALABAC: (CM 2543–2546) PHILIPPINES: exact locality unknown (MCZ 7806).

Taxonomic notes.—The description of *Dipsadomorphus d. multicinctus* was based on four specimens, one of which, from an unknown Philippine locality, was shown to belong to a population of *Boiga dendrophila* from Luzon Island named by Taylor as *Boiga dendrophila divergens* [Brongersma (1934) 217]. The three other syntypes had been obtained by A. Everett on Palawan Island. Brongersma [(1934) 217] restricted the type locality of Boulenger's variety "*multicinctus*" to Puerto Princesa, Palawan Island, thereby fixing the name to apply to that population.

Diagnosis.—Ground color black, with 58 to 84 narrow yellowish crossbars on the body; ventrals 216 to 240. Standard length: ♂ 890, ♀ 950; tail length: ♂ 281, ♀ 302.

Descriptive notes.—Head black above, with or without yellowish spots; nuchal bar occasionally reaching parietal; upper labials yellowish, edged with black, sometimes bars fusing across supralabials dividing yellow space into upper and lower portion; gular shields yellow, edged with black; 58 to 84 narrow yellowish crossbars on dorsum terminating on ventrals; 21 to 29 yellow bars on tail; venter black with ventrolateral series of yellow bars, continuations of dorsal crossbars; ventrals 216 to 240; subcaudals 105 to 117; usually 8 upper labials, third, fourth and fifth bordering orbit; nasal divided; temporals 2 + 3, rarely 3 + 3. [In part after Brongersma (1934) 246–247.]

Sexual dimorphism.—The sexes differ in the average of ventral shields, in the total of ventral plus subcaudal (vertebral) count, and in the ratio of tail length/standard length (Table 5). The principal differences between sexes are found in the dimensions of the body rather than the tail. Indeed, it is interesting to note that the differences in subcaudal counts between sexes are very slight compared to the differences in ventral count. Furthermore, the ratio of tail length/standard length is larger among the four females than the average for five males, indicating a longer tail in the female. The significance of this inversion of characters is not understood.

TABLE 5.—*Summary of variation between sexes in Boiga dendrophila multicincta.* *

Character	Male			Female		
	N	Mean	Range	N	Mean	Range
Ventrals	7	224.0	216–233	9	232.1	224–240
Subcaudals	7	112.4	107–117	9	111.4	10[illegible]–117
Ventrals plus subcaudals	7	332.6	328–348	9	342.3	333–350
Tail length/standard length	5	0.288	0.262–0.309	3	0.300	0.287–0.318
Crossbars on body	4	69.8	58–80	4	78.3	71–84
Crossbars on tail	2	24.5	23–25	4	24.8	23–26

* Includes data given by Taylor (1922a) for specimens not examined in this study.

Females average slightly more crossbars on the body than males, although there is no difference in the range. The number of bars on the tail is the same for both sexes.

BOIGA SCHULTZEI Taylor.

Boiga schultzei TAYLOR (1923) 552, pl. 3, fig. 3 (type loc.: Palawan Island: type in Museum of Comparative Zoology, Harvard College; original description, comparison with *B. drapiezi* and *B. angulata*).

Boiga drapiezi LEVITON (1963) 400 (listed from Palawan Island).

Range.—(For Philippines only.) PALAWAN: without exact locality*).

Material examined (*2*).—PALAWAN: without exact locality (MCZ 25791, holotype; CAS 62153, paratype).

Taxonomic notes.—In 1923, Taylor described *Boiga schultzei*, based on two specimens from Palawan. It is difficult to separate Taylor's species from typical *B. drapiezi*. The two nominal forms seem to differ primarily in color pattern. Taylor [(1923) 553] stressed that *B. schultzei* differed from *B. drapiezi* in having "two instead of three labials entering the orbit; there are seven instead of eight upper labials; there

is a well-developed loreal present." However, of the two specimens Taylor had before him, both of which I have seen, one has seven upper labials and one eight. There are seven upper labials on the type specimen, the third and fourth bordering the orbit; the paratype has eight upper labials (on both sides, not on one side as indicated by Taylor [(1923) 553], and the fifth and sixth shields border the orbit. As Taylor pointed out, only two labials border the orbit; however, in *B. drapiezi* either two or three shields border the orbit. Taylor also suggested that the presence of a loreal will distinguish the two species, but the loreal is either present or absent in *B. drapiezi* (present in the one specimen of the Bornean population I have seen). Indeed only in color pattern do the two forms appear to differ, and even this is not great. In one of the two color patterns of *B. drapiezi* (see discussion under *B. angulata*) there is a series of narrow dark and light crossbars, numbering about 50; in *B. schultzei* there is a series of more than 70 narrow dark crossbars, each a scale row in width, separated by three to four rows of lighter scales.

Additional comments made under *B. angulata* apply here too, and it is necessary to wait until *B. drapiezi* is more clearly defined before it is possible to take a position on *B. schultzei.*

Diagnosis.—Scales in 19 longitudinal rows at midbody; loreal present or absent; preocular reaching upper surface of head; posterior chin shields equal to or longer than anterior pair; dark crossbands on dorsum, very narrow; a narrow dark line present along lateral side of ventrals; hemipenes spinose. Standard length: 956 mm [♂]; tail length: 370 mm [♀].

Descriptive notes.—Maxillary teeth 16; anterior maxillary teeth not strongly enlarged; 1 preocular, reaches upper surface of head and frequently in contact with frontal; loreal present or absent; 2 postoculars; temporals 2 + 2, 2 + 3, or 3 + 3; usually 8 upper labials, rarely 7, the third, fourth and fifth or rarely third and fourth, fourth and fifth, or fifth and sixth shields bordering orbit; anterior and posterior chin shields about equal; ventrals 250 to 276; subcaudals 114 to 163; anal plate single.

Hemipenes extends to eighth subcaudal plate, unforked; sulcus spermaticus unforked; spinose throughout, the spines gradually decreasing posteriorly; a few shallow calyces at distal end.

Color (in alcohol) light brown above with numerous darker brown transverse spots or narrow crossbars on dorsum; light brown below with numerous minute spots of dark brown and two usually distinct dark brown stripes extending the length of the body.

BOIGA PHILIPINA (Peters).

Dipsas philippina PETERS (1867) 27 (type loc.: Ylaces [=Ilocos Province, *fide* Taylor (1922a) 206], Luzon Island; type in Berlin Museum; original description); BOETTGER (1886) 113 (listed).

Dipsadomorphus philippinus, BOULENGER (1896) 77 (description after Peters).

Boiga philippina, GRIFFIN (1911) 263 (listed in key); TAYLOR (1922a) 206 (description after Peters).

Range.—LUZON: Ylaces [= Ilocos Province].

Material examined.—None.

Taxonomic notes.—This species is known from the type specimen alone. I have not seen that individual. Based on the original description, it would seem that the species differs from *B. angulata* in the size of the anterior palatine teeth, in having two preoculars, and in the lower number of ventral counts. Boulenger [(1896) 77] states that it is "Like *D. irregularis*, but preocular divided." Boulenger neglects to point out that there are fewer scale rows than usual for *B. irregularis*. I suspect that this nominal species will be found to be conspecific to *B. angulata*.

Diagnosis.—Two preoculars, upper shield nearly in contact with frontal; anterior palatine teeth said to be greatly enlarged; scales in 19 longitudinal rows at midbody. Standard length.—435; tail length 155. (Ventrals 240, subcaudals 133.)

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